



PUPATION SITE SELECTION  
IN THE STABLE FLY,  
(DIPTERA: MUSCIDAE)

by

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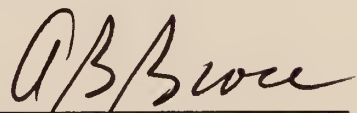
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## LITERATURE REVIEW

Stable flies, *Stomoxys calcitrans* L., are an important economic pest in many areas of the United States, in feedlots and dairy operations. Economic losses to the livestock industry in the U. S. due to the stable fly have been estimated by the USDA/ARS to have been \$814 million per year during 1973-1975, of which \$735 million represented losses in beef cattle. Stable flies have been shown to cause reduction in weight gains and feed efficiency in growing and finishing cattle (Campbell et al. 1977). A number of investigators have reported decreases in milk production in response to stable flies (Bishopp 1913, Freeborn et al. 1925). Stable flies feed mostly on the legs of cattle, causing the cattle to stamp their feet and seek water to avoid the flies. (Bishopp 1913, Hoffman 1968, Bidgood 1980). It is known that stable flies cause cattle to bunch and increase their activity, which in combination causes heat stress to the cattle. This condition produces cattle of lower weight, and of lower quality.

In addition to causing reductions in weight gain and decreases in milk production, the stable fly is an annoyance to both humans and animals, and is capable of transmitting a number of diseases. Bishopp (1913) reported that stable flies were capable of transmitting surra, souma, anthrax, and infectious anemia. Stable flies also act as a mechanical vector of leishmaniasis, brucellosis, swine erysipelas, fowl pox and anaplasmosis (Hall and Smith 1986). The stable fly can also act as an intermediate host for *Habronema* species (a common stomach worm of horses)

and the dog heartworm, *Dirofilaria immitis* (Hall and Smith 1986).

Adult female stable flies require a blood meal prior to oviposition (Bishopp 1913, Venkatesh and Morrison 1980). Hafez and Gamal-Eddin (1959) reported that under laboratory conditions, females will take a full meal after copulation. When the eggs are mature the mated female will seek an appropriate oviposition site. The female crawls into the substrate in search of an oviposition site using olfactory and tactile cues. Adult females are generally known to be highly thigmotactic, causing the fly to lay eggs in cracks and crevices along the substrate. Hoffman (1968) observed stable fly females crawling through piles of straw and manure in search of an appropriate oviposition site. Prior to ovipositing the female everts the ovipositor and drags it across the surface of the substrate.

Stable flies are oviparous and will oviposit from 10-86 eggs per batch, depending on the season (Hafez and Gamal-Eddin 1959). Mitzmain (1913) reported that females lay up to 20 batches of eggs, totaling 632-820 eggs per individual. Parr (1962) documented that females averaged 300-400 total eggs laid in 10-12 separate batches. The flies in Parr's study also had a considerably faster development time than those in Mitzmain's study. Hoffman (1968) indicated that total eggs per stable fly varied greatly, ranging from 22 to 307, with an average of 183, and 79.95 % of the eggs hatching.

The first complete documentation of the life cycle of the stable fly was conducted by Newstead (1906). Since then a number of studies have been conducted



on the life cycle, bionomics and behavior (i.e. Bishopp 1913, Mitzmain 1913, Parr 1962). Parr (1962) reported the completion of the life cycle from egg stage to adult at 80°F and 80% RH to be 12 days. Newstead (1906) reported the life cycle at 72° F (with 65° F at night) to consist of an egg incubation period of 2-3 days, larval stage lasting 14-23 days, and pupal stage from 9-13 days, resulting in a total life span of 25-39 days, considerably longer than that reported by Newstead (1906). Mitzmain (1913) documented that stable flies had an egg incubation time of 10-26 hours (under temperatures of 30°C), a larval period ranging from 6-26 days, or 7-8 days under optimal conditions, and a pupal period of 5-6 days. Bishopp (1913) stated that the life cycle could be completed from 19 days up to 43 days. He stated that the development usually averaged 21-25 days under favorable conditions; remaining fairly consistent with the results of Newstead (1906). Sutherland (1979) found an optimal temperature for egg incubation to be 30°C, with a viable range from 10-40°C; an optimal range for pupal and adult survival from 20-30°C.

Hoffman (1968) reported a smaller range for larval development; 216 to 384 hours under varying temperatures. He also indicated that larvae could withstand a great deal of submersion under water; demonstrating that 79 % of larvae immersed for 17 hours eventually pupated. Newstead (1906) reported that the larval stage normally lasts 14-21 days under favorable conditions, but it is lengthened by low moisture and exposure to light, lasting from 31-78 days. The pupal stage is also



highly tolerant to water, and could withstand up to 142 hours immersed in water and still emerge (Hoffman 1968). Pupal development does not progress during the time the pupa is immersed, consequently prolonging the pupal stage.

Stable flies have been found to breed in a wide variety of habitats. Bishopp (1913) reported stable fly breeding in straw stacks remaining after harvest. Scholl et al. 1981 observed open silage storage systems to contain large numbers of immature stable flies. The majority of larvae were found in corn silage within ca. 5 m of the moist silage-soil interface along the edge of the pile. Open bunker-type silos contributed to the large numbers of stable flies at dairies in Michigan (Guyer et al. 1956). Simmonds and Dove (1941) reported that stable flies breed extensively in marine grasses deposited along the shores of bays and other inlets of north-western Florida. Certain mixtures of bay grasses also provided a highly suitable breeding media (Dove and Simmonds 1942).

Williams et al. 1980 sampled a number of potential breeding sites in Florida and concluded that decomposing silage and green chop (freshly cut sorghum) were the most prolific breeding sites for stable flies. Pondgrass and eelgrass were also among suitable larval breeding media. Hall et al. 1982 reported stable flies breeding in large round hay bales. Immatures were mainly found in the hay bale-substrate interface where moisture had accumulated. Fermenting hay bales proved to be very attractive for larval breeding.

Stable flies have been found to overwinter as third instar larvae, pupae, and

occasionally as adults (Simmonds 1944). Larvae and pupae survive the winter in breeding media that do not freeze over the winter. Open trench silos provide a suitable overwintering site due to the relative warmth generated by the fermentation and decomposition processes (Scholl et al. 1981, Hall and Smith 1986).

Hafez and Gamal-Eddin (1959) observed that in the cold season female stable flies oviposited inside stables in animal beddings consisting of manure, straw, food remains, fodder and urine. In the warm season the female oviposited on cattle and horse manure mixed with straw and urine. Pure cow, donkey, buffalo, and horse manure did not attract female stable flies for oviposition, however mixing with straw and urine rendered the manure attractive for oviposition (Hafez and Gamal-Eddin 1959, Parr 1962). In contrast, Newstead (1906) and Mitzmain (1913) reported that stable flies do breed in pure horse and cattle manure, as well as in chicken excrement (Coffey 1966). More recently, Sutherland (1978) confirmed that stable flies could not breed in pure chicken excrement, however when mixed with *Pinus* spp. sawdust, chicken excrement supported larval breeding. Sutherland (1978) also bred larvae on cattle, donkey, goat, horse, pig and sheep manure, with varied success.

Parr (1962) concluded that three conditions were usually needed for suitable stable fly breeding, including, the presence of rotted cattle manure, the presence of rotted straw, grass or leaves, and shade for reducing temperature and preserving moisture (breeding sites usually contained urine as well). The basic elements of

these conditions seem to be decaying vegetable matter, sufficient moisture, and the presence of microorganisms (from manure).

Haas (1986) described the biological succession of aging manure. In aging manure, various physicochemical changes occurred over time, which she speculated was in part due to differences in microbial populations. These changes were presumably the cause of the larval succession observed in aging manure. Haas (1986) indicated that Sarcophagid larvae colonized fresh manure, followed shortly by house flies, then Stratiomyiids, and lastly, by stable flies. Over time, the manure became more attractive to gravid female stable flies, hence their late appearance in manure succession. She attributed the appearance of stable fly larvae late in aging manure to female preference, rather than the inability of larvae to survive in fresh manure. Haas (1986) speculated that larval competition may have been a contributing factor to the late appearance of the stable flies. She stated that this phenomenon could be a function of the adult gravid females' attraction to chemical cues which appear relatively late in the manure aging process. These attractants may be critical for timing of oviposition and larval breeding in other preferred media.

A number of researchers have attempted to correlate various parameters with either adult visitation or presence of immatures. As early as 1913, Fuller reported an unusual outbreak of adult stable flies directly following floods in South Africa. Hall et al. 1983 also linked high stable fly populations in pastures to heavy rainfall

in early summer in Missouri.

Gilbertson and Campbell (1986) were unable to correlate immature stable fly presence with pH, total and volatile solids, inorganic material, electric conductivity or temperature. In an earlier study, Rasmussen and Campbell (1981) could not correlate larval presence with pH, organic matter or moisture content. Haas (1986) reported that appearance of fully gravid female stable flies showed no significant correlation with a number of environmental parameters studied: pH, CO<sup>2</sup>, ammonia, moisture or temperature. Rasmussen and Campbell (1981) found immatures in the field at an average moisture content of 51.98% and a range of 22.9 to 65.02%; an average organic matter of 35.74 % with a range of 21.37 to 51.73%; a pH of 7.69 with a range of 7.13 to 8.23; and an average temperature of 23.46°C with a range of 21.1 to 25.25°C. Gilbertson and Campbell (1986) found immatures in the field from a range of 8.6 to 34.6°C; and at pH values greater than 7.

A number of recent studies concerning the stable fly have attempted to describe the physical characteristics of common stable fly breeding sites. Fencelines and gates proved to be the most common breeding sites in a study in northeast Nebraska (Gilbertson and Campbell 1986). Electric fences were particularly good for larval breeding since cattle avoided these fences allowing manure to accumulate near the fencelines. Other important sites included waterers, animal lot areas, drainage areas and, to a lesser extent, silos. Meyer and Petersen (1983)

categorized 16 different types of breeding sites for house fly and stable fly pupae during their study of feedlots and dairies in eastern Nebraska. They reported that in small feedlots, fenceline, where soil and manure would accumulate, was the major source of stable flies, followed by drainage ditches, where manure would mix with rain water. Other notable sites were empty lots, where manure would remain and crust over; haylage, freshly cut alfalfa stacked or improperly stored in silos; potholes, lower areas avoided by the cattle; and spilled feed. In contrast, in large feedlots spilled feed was the most prolific breeding site for stable flies, followed by fresh, unstacked corn silage. Haylage, old corn silage, soiled straw bedding and fenceline manure supported the remainder of fly breeding. On dairy operations, stored manure was the most important stable fly breeding site, followed by soiled straw bedding and drainage ditches.

In view of these findings, it becomes obvious that one of the best methods of stable fly control may simply be sanitation coupled with awareness of potential breeding sites. Gilbertson and Campbell evaluated beef cattle feedlot operations and illustrated the importance of feedlot design and management practices in controlling fly breeding (1986). A number of other more costly control methods have also been employed. However, as stable flies became increasingly resistant to insecticides, such as DDT and organophosphates (Georghiou and Taylor 1976), other insecticides and/or methods were needed. Fairly recently a number of novel techniques have been tested for control of the stable fly, such as the use of the



sterile insect technique (Ramsamy 1977, Williams et al. 1977, Easton 1986), use of chemical repellents (Roberts et al. 1960), release of *Spalangia endius*, a hymenopteran parasitoid as a biological control agent (Morgan 1980), and visual attractants (Williams 1973, Agee and Patterson 1983).

Gilbertson and Campbell (1986) indicated that stable fly and house fly immatures were rarely found in the same area. Of samples containing immatures, only 10 % contained both stable flies and house flies, implying some element of habitat separation. Haas (1986) found that different species of flies colonized manure at different times during its succession. This may be a potential mechanism for habitat separation. Rasmussen and Campbell (1981) noted that competition between stable flies and other insects (including the house fly) may be one of the reasons for the differential colonization. The role of competition has been studied in a number of coprophagous larvae, such as face fly (Moon 1980), bush fly (Ridsdill-Smith et al. 1986), house fly (Bryant 1969), and in *Drosophila* (Manning and Markow 1981), but has not been examined closely in the stable fly. In these studies, competition at high densities resulted in lower weights and lower survival.

As early as 1885, Pasteur speculated that intestinal bacteria are essential for survival of higher animals (Glaser 1924). A number of dipteran larvae may be dependent in some manner on the presence of microorganisms. Glaser (1924) carefully concluded that "dipteran larvae (including *Stomoxys calcitrans*) are dependent upon certain growth factors that are destroyed by certain high tempera-

tures ordinarily used in sterilizing media. These growth factors can be replaced in the diet by contaminating the media with almost any microorganism that grows well on the substrata." Glaser did not conclude that the microorganisms themselves are essential because of the lack of specificity of bacteria or yeasts needed to stimulate larval growth. The addition of live bacteria or yeasts, as well as the addition of large quantities of dead bacteria or yeasts killed at low temperature, or the addition of sterile animal tissue extracts or sterile plant juices also supported larval growth. Hollis et al. 1985 found that face fly development in steam sterilized bovine manure was significantly retarded. Mean pupal weights were significantly lower, and egg-to-pupa survival was decreased. Eleven bacterial isolates were added singly and mixed together to sterilized manure. Single bacteria improved larval development, however the mixed inoculum allowed for normal growth.

More recently, Lussenhop et al. 1986 reported that in the laboratory, *Neomyia cornicina* larvae increased bacterial turnover and decreased bacterial numbers as larval density increased. In the field, larvae were associated with higher bacterial densities. Lussenhop et al. 1986 assumed that individual larvae would benefit not from high bacterial numbers, but from high bacterial turnover. Since high densities of larvae increased bacterial turnover, they predicted that pupal weights would increase at high larval densities. However, Lussenhop et al. 1986 was unable to show an increase in pupal weight in response to bacterial density. In fact, pupal



weights decreased with increasing bacterial density. They concluded that the increase in bacterial turnover was insufficient to benefit individual larvae within the feeding aggregation.

Several authors have tried to correlate fly larval density with increased pupal weights or increased survival with little success. Valiela (1969) conducted competition experiments on face fly larvae by subjecting them to various coleopteran and dipteran predators. He found a reduction in pupal weight at high larval density. At lower densities, pupal weights also decreased, however there was also higher mortality. Valiela speculated that a "critical minimal density is needed for the proper liquefaction of the substrate to make available enough food for the filter-feeding larvae." Black fly larvae are also filter-feeders, and are known to have a very low assimilation efficiency of ca. 2% (Wotton 1978). Wotton (1980) speculated on the importance of coprophagy in aggregations of black fly larvae. He suggested that by feeding on feces (of other larvae and its own feces), larvae were able to utilize or assimilate the nutrients more efficiently. Increased contact of the food materials in the gut of larvae with enzymes and intestinal bacteria increases the overall feeding efficiency. Zvereva (1986) discussed the same phenomenon in house flies in relation to more efficient food utilization.

Sullivan and Sokal (1963) have also studied the interaction of density and larval performance. They noted the effects of "undercrowding" in their study on the effects of density on several house fly strains. Two of the strains showed

either decreased weight or decreased % emergence in response to undercrowding (densities less than 40 larvae in a half-pint jar containing 35 g of artificial medium). At low density, larvae may not be able to compete with mold and fungus which often ties up the nutrients by presenting physical barriers (i.e. mycelium of the fungus) to the larvae (Zvereva 1986). However, they estimated that the effects of undercrowding were relatively unimportant overall (Sullivan and Sokal 1963). However, Zvereva (1986) demonstrated that individual house fly larvae had lower survival than individuals developing in a group. In addition, individuals in small groups (1-5 larvae) were less viable, and had lower pupal weights than those in large groups. Under unfavorable conditions, these differences were magnified. Zvereva also demonstrated high competition between mold/fungi and house fly larvae, and that large groups of larvae are better able to restrain further growth of the mold/fungi; individual larvae were shown to be less viable and in some cases, could not survive at all.

Given the idea that large aggregations of larvae may feed more efficiently than small groups, Zvereva (1986) examined the role of adult behavior in affecting aggregations of offspring. Using dead, pinned adult house flies, Zvereva (1986) demonstrated that adult females were more attracted to and laid more eggs on a surface on which other individuals were located. He also suggested that flies laid eggs more readily on a surface containing house fly eggs. Thus, this process of laying eggs together may promote aggregation of larvae in large groups. Bidgood

(1980) reported a similar phenomenon in adult stable flies, which were more attractive to patterns with other adults on that pattern. Hoffman (1968) observed this same phenomenon in stable flies, however he speculated that communal egg laying was a function of environmental stimuli (i.e. physical and chemical attributes of the substratum) rather than an association with other females. Regardless of the mechanism which brings gravid females together, the effect is the same; large aggregations of larvae with potential advantages.

There are advantages to existing in an aggregation, such as increased resistance to desiccation and high temperatures (Allee 1931). This has been well documented in the house fly by Zvereva (1986), who also claimed that groups of house flies can manipulate the microhabitat of the breeding media in a favorable way. However, with higher densities there may be increased competition for food, moisture and space, along with an increased visibility to predators, and favorable conditions for spreading diseases and parasites (Sullivan and Sokal 1963, Zvereva 1986).

Immelmann (1980) discussed the differences between an "aggregation" (or a "collection") and a "true group." In an aggregation, animals do not seek out each other, but simply orient to similar environmental conditions. The grouping is considered to occur accidentally. However, a true group is a more complex grouping and cannot be explained by similar conditions. A true group implies that the animals may be orienting to each other, and the grouping may serve some adap-

tive function. House flies and stable flies may form true groups, although there is no conclusive evidence supporting this fact (with the possible exception of Zvereva 1986). Although the proximate mechanism may differ in these two types of groups, the ultimate cause or benefit of existing in an aggregation may be the same.

McFarlane and Alli (1986) noted that larvae of the German cockroach, *Blattella germanica*, show arrestment under favorable conditions, followed by an attraction to other larvae. The cotton stainer, *Dysdercus intermedius*, aggregates in response to both visual and olfactory cues on cotton seeds (Youdeowei 1969). Zvereva (1986) reported that house fly larvae formed groups in uniform media. In addition, house fly larvae selected media which had previously contained other house fly larvae or fresh media inoculated with a water extract from media previously used by house flies over the control media. However, this may be due to the presence of bacteria and other by-products of the larvae.

There is, however some controversy as to the distribution of stable fly larvae and pupae in the field. During their evaluation of feedlots in northeast Nebraska, Gilbertson and Campbell (1986) reported that stable fly larvae and pupae were never found clustered, but were dispersed in various habitats. However, Parr (1962) claimed to discover "seething masses of *Stomoxys* larvae and also pupae." The significance of forming aggregations in stable fly larvae and/or pupae remains unclear.

At the end of its last larval instar, holometabolous insects enter into a wandering phase, which is characterized by increased locomotor activity, purging of the gut contents, negative phototaxis, and exposure of the aorta through the epidermis. Some or all of these physiological and behavioral changes may occur in dipteran larvae (Zdarek 1985). These behavioral changes are hormonally mediated (Fraenkel 1934, Truman and Riddiford 1974), hence the ability to manipulate the wandering phase timing and/or length may be limited.

The lengths of the wandering phase for diptera are quite variable. Finlayson (1967) reported that *Glossina morsitans* had wandering lengths in the range of 1.4 h to 6 h, the latter time being rare. Under dark conditions, time from beginning of wandering to the barrel stage for *G. morsitans* was as short as 23 min. *G. morsitans*, however are viviparous, quite different from other diptera in that larvae feed from milk glands in the adult uterus, and are laid after the larvae have completed feeding (Finlayson 1967). Essentially they are laid at the beginning of their wandering phase, and they begin to burrow into the ground immediately (Finlayson 1967). *S. bullata* spent 40-60 h in the wandering stage (Zdarek 1985). Denlinger (1985) claimed that non-diapausing *Sarcophaga bullata* wander for 24-48 h, but that diapausing strains spent over two weeks in the wandering phase.

There appears to be tremendous plasticity in the initiation of puparium formation. Saunders (1975) demonstrated that high moisture would increase the length of the wandering period in *S. argyrostoma*. Exposure to CO<sub>2</sub> anesthesia was



found to delay puparium-formation in *Drosophila melanogaster*, however, Saunders (1975) found that CO<sup>2</sup> exposure decreased the wandering phase for *S. argyrostoma*, and that removing larvae from its food source 3-4 days early shortened the mean length of larval development. Zdarek and Fraenkel (1972) showed a similar delay in pupariation for *S. bullata* under conditions of high moisture. Ohtaki (1966) found that both *Aldrichina grahami* and *Calliphora lata* delayed pupariation under high moisture conditions. *Sarcophaga peregrina* showed a delay in pupariation under high moisture conditions in both laboratory and field conditions (Ohtaki 1966). However, pupariation in *S. peregrina* was delayed over 100 h when the larvae were in wet rearing conditions. Ohtaki (1966) claimed that *S. peregrina* remained as "mature larvae for ten days or more without pupation." From this description, it is difficult to differentiate between the length of the larval period from the actual length of the wandering phase. Many of these terms are poorly defined in the literature. *Stomoxys calcitrans* also seems to be capable of delaying pupariation to some extent, however survival is very low after being subjected to high moistures for long periods of time (Hoffman 1968).

In artificial selection experiments for early and late pupariation, Bradley and Saunders (1986) demonstrated that two strains of *Sarcophaga argyrostoma* differed in their larval wandering time, and that they could select for "fast" and "slow" pupariation. They concluded that control of pupariation was polygenic.

Groups of 25 *Calliphora erythrocephala* pupariated significantly later than

did individual *C. erythrocephala*. Berreur et al. 1979a found that individual insects placed in rotating flasks in order to receive continual mechanical stimulation also delayed pupariation significantly compared to undisturbed individuals. Groups of undisturbed insects and individuals undergoing constant stimulation were not significantly different in their mean times to pupariation. This indicates that the effect of density on pupariation was due to the mechanical stimulation by nearby larvae. However, Finlayson (1967) demonstrated that *G. morsitans* pupariated sooner in groups of ten placed in small one inch caps containing sand than single larvae placed in thin plastic tubing. Larvae in plastic tubing (with mechanical stimulation on all sides) pupariated sooner than larvae kept in small one in caps (with much less mechanical stimulation). Finlayson (1967) found that mechanical stimulation seemed to decrease wandering length which contrasts the results from Berreur et al. 1979a.

In *Sarcophaga argyrostoma*, the initiation of larval wandering (termed exodus behavior) occurred as a gated circadian rhythm (Richard et al. 1986). The majority of larval wandering occurred at night. Richard et al. 1986 discussed possible advantages to the gated nocturnal wandering behavior, such as avoidance of desiccation, or diurnal predators. However, this would not free larvae from nocturnal predators. In contrast to larval exodus, formation of the puparia was not rhythmic (Richard et al. 1986). Pupal eclosion was also under control of a gated circadian rhythm (Saunders 1976), as was pupal eclosion in the stable fly



(Hoffman 1968).

Richard et al. 1986 demonstrated that *S. argyrostoma* delayed pupariation under certain light/dark cycles up to a certain point, before showing a phase-jump back to an earlier transition. With varying night lengths, larval exodus showed 2 or 3 clear peaks, where most to all activity occurred in the dark, except when night lengths became too short, and activity would continue into the succeeding light phase. Under conditions of continuous light, wandering behavior was arrhythmic.

Wandering behavior is presumably induced by ecdysteroids (Fraenkel 1934). The release of prothoracicotropic hormone from the brain (initially stimulated by an earlier release of ecdysone), is thought to be the gated event controlling larvae wandering (Truman and Riddiford 1974, Richard et al. 1986). Berreur et al. 1979b demonstrated that a small peak of ecdysteroid existed in the haemolymph prior to a larger peak associated with puparium formation. Berreur et al. 1979b suggested that this smaller peak was involved in the timing of pupariation. Under adverse conditions, the small peak is delayed as is the larger peak (Berreur et al. 1979b). The secretion of ecdysteroid is suggested to be caused in some way by favorable surroundings (Ohtaki 1966, Zdarek and Fraenkel 1970). Zdarek and Fraenkel (1970) demonstrated that *S. argyrostoma* became more sensitive to ecdysone injections the longer they had been exposed to dry (favorable) conditions after being subjected to high moisture (unfavorable) conditions. It is not known

how labile the system might be after the small peak of ecdysteroid (implicated in the timing of pupariation by Berreux et al. 1979b) is released by the insect.

Zdarek (1985) hypothesized that delayed pupariation was an adaptation to food scarcity. Zdarek (1985) determined that a minimum amount of feeding after the last instar larval molt was necessary for pupariation to occur in *Calliphora vomitoria*. The mechanisms for the larval-pupal molt are irreversibly activated at some time by this brief period of feeding. If larvae are undernourished, this post-feeding period is longer. The post-feeding period refers to the period after feeding stops and before the insect undergoes the behavioral switch to wandering. This post-feeding stage specifically refers to conditions of food scarcity, where a last instar larvae may not have fed sufficiently to reach an optimal weight. However, with no food available, it is advantageous for the insect to begin pupariation because over time the insect may lose weight. This situation is analogous to extreme environmental conditions. Under flood or drought conditions, there may be a post-feeding stage which is delayed by extreme conditions. For example, under drought conditions, pupariation is delayed while the larvae are searching for a more suitable pupation site, yet eventually the insect will pupariate, as the pupal stage is known to conserve more water than the larval stage. An important condition to this idea is that the wandering larvae need to be able to travel some distance to search for more optimal conditions. *G. morsitans*, for example, was found to travel from 41-190 cm depending on the light conditions in the labora-

tory (Finlayson 1967), while wandering *S. bullata* larvae traveled more than 20 m under lighted conditions (Zdarek 1985). This may or may not be sufficient to allow the larvae to 'choose' a suitable site from among the limited (spatially and temporally) sites available.

*S. argyrostoma* pupae reared under short days and high temperatures, or reared at low temperatures, showed an arrestment of pupal development, often termed diapause (Fraenkel and Hsiao 1968). Diapause or the arrested development could be quickly terminated by an increase in temperature (Fraenkel and Hsiao 1968, Gibbs 1975), chilling (Fraenkel and Hsiao 1968), and hexane treatment (Denlinger et al. 1980, Lee and Denlinger 1985).

Sokal et al. 1960 suggested that pupation site selection may be a function of relatively early larval influences. Sokolowski and Hansell (1983) demonstrated that two separate strains of *Drosophila melanogaster* pupated at significantly different heights, suggesting that there is also a genetic component to site selection. Pupation height was also affected by larval density. Bauer and Sokolowski (1985) illustrated differences in pupation height in a natural population of *D. melanogaster*, dispelling the belief that pupation height may have been an artifact of laboratory strains, particularly genetic mutants.

It has been reported for *D. melanogaster* that a number of behavioral characteristics such as pupation distance from the food source, pupal microhabitat selection (i.e. on or off host fruit), and pupal microhabitats within host fruit are geneti-

cally based (Sokolowski 1985). *D. melanogaster* have also been known to show pupation behavior differences in selecting peripheral pupation sites versus central pupation sites (Sokal 1966). Selection experiments carried out for 17 generations of two lines of *D. simulans* indicated a genetic component to this pupation behavior (i.e. peripheral versus central pupation site selection (Ringo and Wood 1983). This suggests that pupation site selection is not rigidly controlled, and will respond to certain selection pressures. In addition, a number of these parameters had an impact on survival to the pupal stage (Sameoto and Miller 1967). Presumably, stable fly larva will respond to a number of these similar parameters prior to pupation, thus enhancing its fitness.

The behavior and ecology of immature stages have often been overlooked. The proficiency of larval feeding has an obvious impact on its growth rate, development time, pupal and adult weight, dispersal ability, probability of survival, and in addition, the overall fitness of the organism (Slansky and Scriber 1985). For example, reduced growth in the larval stage may have an impact on adult fitness in terms of size, mating and reproductive success, fecundity, quality of offspring, etc.

In addition, an optimal pupation site is important for the successful development of the organism to eclosion. The insect is highly susceptible during the pupal stage to predation, parasitism, and to the effects of physical factors. Although much is known about stable fly larval breeding sites, little has been

documented on pupation sites and the behavior leading to the selection of an optimum or suitable site to insure the insect's survival.



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## STATEMENT OF OBJECTIVES

Much is known about suitable larval habitats for the stable fly, *Stomoxys calcitrans*, however very little is known about pupal sites. Pupae are not encountered in the field as often as are larvae, and virtually nothing is known about the process of selecting a pupation site. The stable fly is an important economic pest throughout the world and further knowledge of the pupation sites may be useful in devising control strategies. Because of this lack of information, the behavior of pre-pupal larvae was studied to provide some information in this area.

The relationship of parameters selected by wandering larvae in laboratory assays was compared to actual pupation sites in the field (Chapter 1). Prior to choosing a pupation site larvae enter a wandering phase. The process of wandering was also examined (Chapter 2) to determine its relationship and relative importance in the selection of pupation sites. The wandering process is hormonally mediated, hence larvae may be able to manipulate (or delay) the timing and extend the length of the wandering phase. This ability will directly impact the ability of the larvae to choose a suitable pupation site. Very little is known about the processes of wandering and pupariation in the stable fly. This study investigated the wandering phase length, and environmental factors which affected the wandering phase.

**PAPER 1: Environmental Components of Pupation Site Selection of the Stable Fly, *Stomoxys calcitrans* (Diptera: Muscidae)**

**To be submitted to Environmental Entomology**

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ABSTRACT. The effect of moisture, temperature, light, pH, and osmolality on pupation site selection and survival of stable fly pupae, *Stomoxys calcitrans*, was evaluated by means of choice tests. Of the moisture levels tested, 50, 68, 89%, more pupae were found at 68%. Larvae pupated in the dark when placed in a light gradient, and at a temperature of ca. 27° C., when placed in a temperature gradient. When given a choice of pH levels, 5.2, 6.4, 7.2, 9.3, stable flies pupated at the highest pH. Conversely, insects pupated at the lowest osmolality when given a choice of 111, 254, 403, 609 mmol/kg. Measurements of pH and osmolality of field habitats were found to be negatively correlated. A discriminant analysis of the parameters measured in the field indicated that pH was the most important variable in categorizing pupal sites versus non pupal sites. In texture choice tests, larvae pupated in rearing media containing sand over media without sand. In the laboratory, substrate density had an effect on the vertical distribution of pupae; in all density treatments, 82% of pupae were found in the top 4 cm in a 12 cm column of substrate. Distribution of pupae in uniform media was found to be non-random. Field measurements of naturally occurring pupation sites demonstrated that pupae were found in a broad range of the above habitat parameters. Larvae released in the field dispersed up to 911 cm. When released into the field, laboratory reared larvae did not appear to relocate under more 'suitable' conditions. In summary, larvae tested in the laboratory selected very narrow ranges of each tested parameter, yet in the field, pupae were found in a broad range of the same parameters, This implies that other selection pressures aside from the

measured environmental parameters are important in the selection of pupation sites.

**KEY WORDS** Insecta, stable fly, pupation, environmental parameters

## INTRODUCTION

THE STABLE FLY, *Stomoxys calcitrans* L., is an important economic pest throughout the world, and causes severe economic losses to the livestock industry (Campbell et al. 1977). It is commonly considered to be an annoyance to humans as well as to animals. Thus far, biological control measures (e. g. the use of hymenopteran parasitoids) have proven to be only marginally successful (Morgan 1980). Although the use of pesticides is still the most common method of control used today, the most effective control method is the elimination of larval breeding sites.

Stable fly larvae breed in a wide range of moist, semi-aquatic habitats. The most common sites is moist silage that is spilled in the vicinity of feeding and drinking troughs in the feedlot (Scholl et al. 1981, Meyer and Peterson 1983). Other suitable sites include, aged manure (Bishopp 1913), grass clippings (Newstead 1906, Dove and Simmonds 1942), hay stacks (Bishopp 1913), hay bales (Hall et al. 1982), and rotting seaweed (Simmonds and Dove 1941).

Although much is known about suitable larval habitats, very little has been documented about pupation sites of the stable fly. Knowledge of larval and pupal habitat may prove valuable in devising control strategies for this insect pest.

The selection of a suitable pupation site has a direct impact on the organism's developmental time, pupal weight, and survival (Slansky and Scriber

1985). In particular, a suitable pupation site is important for the successful survival of the organism to eclosion. The pupal stage is highly susceptible to predation, parasitism, and to the effects of the environment. The work reported in this paper was undertaken to examine where stable fly larvae select to pupate when presented with different levels of moisture, temperature, light, pH, and osmolality. In addition, the relationship of these parameters selected by wandering larvae in the laboratory were compared to actual pupation sites in the field.



## METHODS AND MATERIALS

## Methods Common to Experiments.

Stable flies were reared at  $25 \pm 2^\circ \text{C}$ ,  $70 \pm 10\%$  RH, and 18:6 (L:D) photoperiod in a vermiculite, wheat bran, fishmeal medium. This medium was used as the substrate in all of the experiments unless otherwise indicated. Stable flies used in all experiments have been reared in our laboratory for approximately 4 years. The stock colony came originally from Lincoln, Nebraska, where it had been in colony for one year. As stable flies near the end of their larval development, they purge their gut, initiating the start of larval wandering. As the larvae begin to wander, their activity level increases, causing them to follow the edges of the rearing pan (30 x 40 cm) and collect in the corners of the pan. For all experiments, wandering larvae were collected from the corners of the rearing pans prior to pupariation. Preliminary studies demonstrated that such larvae would pupariate within 48 h. These larvae are referred to as wandering larvae in this paper.

All choice tests were conducted at  $27^\circ \text{C}$ , in a uniformly lit environmental chamber, and at ca. 70% RH unless otherwise indicated. Light was measured with a light meter to ensure consistent light levels in the chamber. Moisture contents were measured gravimetrically and based on a mean of wet weights taken at the beginning and end of each experiment.

Analysis of variance (ANOVA) of all results were preceded by Bartlett's test of homogeneity of variances (Steel and Torrie 1980, SAS Institute 1987).

Appropriate transformations of the data were made as indicated in each section. Fisher's Protected Least Significance Difference test was used for all multiple comparisons (Steel and Torrie 1980, SAS Institute 1987). Multiple comparisons (LSD) were considered to be significant if  $P \leq 0.05$ .

### Terminology

In the choice tests that are described in the next section, stable fly larvae were given choices of different levels of several parameters such as moisture, pH, etc. In most tests, four different levels of a parameter (such as media at different moisture levels) were placed in pie shaped wedges in a round pan. The wandering larvae were placed in the center of the pan so that they would be exposed to (or at least in close proximity to) all choices (or levels of moisture) at the beginning of the experiment. After 48 hours the number of pupae was recorded in each moisture level. The larvae are very active during the wandering phase and they encounter each moisture level at least once before pupariating. I refer to these as 'choice' experiments, as the larvae crawl through each of the moisture levels (somewhat at random) and eventually pupate in one of these levels. I don't mean to imply that the larvae make a simultaneous sampling of each moisture level and make an active decision about which level they prefer. The terminology in this paper (preference, selection, choice, etc.) is meant to merely indicate that a larva encounters each moisture level as it randomly crawls through the media and eventually ceases wandering and pupates. I have used the term select rather than

prefer, in order to indicate that the mechanism of choosing to pupate at a certain level is not known.

### Effect of Moisture

The pupation site selection in substrates with different moisture contents was evaluated with groups of wandering larvae and individual larvae. In group tests three different mixtures of vermiculite and water were placed in a round pan (19 cm diam), producing 3 moisture levels per pan. The moisture of the mixtures was measured both before and after the experiment. In the first experiment, one high (89%), two moderate (68%), and one low (51%) moisture media were used. Media was placed to a depth of ca. 2 cm in a round pan in wedge-shaped pie sections so that the moderate sections were between the high and low sections. Thirty wandering larvae were placed on the media in the center of the pan and allowed to wander undisturbed. Each pan was covered with clear plastic wrapping and placed in an environmental chamber at 27° C. The number of pupae in each section was recorded after 48 h. This was replicated three times. A second experiment using the same design was conducted with three different moisture levels: 29, 54, and 74%. Data were analyzed as a randomized block design using Fischer's Protected Least Significant difference (LSD) test for multiple comparisons. The data were normalized to combine the two moderate moisture sections as a single treatment. Experiments were also conducted with two low(25%), one high(71%), and one moderate(57%) moisture level; as well as two high, one

moderate, and one low moisture level.

For choice tests with individual larvae, moisture conditions were obtained as described in the group tests. Media of the various moisture levels were placed in small 9 cm petri dishes in 3 wedge shaped pie sections. One wandering larva was introduced into the center of each of 25 dishes. Pupae were counted after all larvae had pupated. Results were analyzed by a chi-square test.

To assess the effect of previous experience of moisture conditions on pupation site selection, three sets of larvae were reared under 3 moisture conditions, 50, 70, and 89% as used in the previous choice tests. Wandering larvae from each moisture level were then tested individually in a moisture choice test as described previously. Results were subjected to a chi-square analysis.

### Effect of pH

The pupation site selection for different pH levels was evaluated with individuals and with groups of wandering larvae. In tests with groups of larvae, media at specific pH levels were prepared by adding phosphate buffer solutions (pH 4, 6, 8, 10 Fisher Sci., Pittsburg, PA) to dry vermiculite to obtain moisture levels of 68-70%. The pH of these mixtures was measured at the beginning and towards the end of the experiment with a digital Corning Model 150, pH meter. The pH values are the means of the initial and final reading; these values were 5.2, 6.4, 7.2, and 9.3. Osmolality (millimoles of dissolved substances per kg of substrate) was determined initially for each pH mixture to ensure that there were no

significant differences in osmolality at different pH levels. Osmolality was measured using 8  $\mu$ l of vermiculite supernatant in a Wescor 5100 C vapor pressure osmometer (Wescor, Logan, UT). Each of the 4 pH/substrates was placed in wedge shaped pie sections in a round (19 cm diam) container. The relative positions of the four mixtures were randomized. Twenty-five wandering larvae were introduced into the center of the arena. Pupae were counted 48 h later. The data were analyzed by Fisher's Protected LSD.

For choice tests with individual larvae, pH solutions (4, 8, 10) were mixed with dry vermiculite as described previously to yield media pH values of 5.7, 7.5, and 9.3. Each pH/substrate was placed in wedge shaped pie sections in small plastic petri dishes (9 cm diam). Individual larvae were introduced into each of the five dishes. The data were analyzed by a chi-square test.

Larval choice tests were also done using only the lower pH levels to determine if the larvae could distinguish among substrates of different low pH levels. Mean pH values of 5.2, 6.4, and 7.4 were used with larva groups, and 5.3, 6.4, and 7.5 were used for tests with individuals. Group tests were analyzed as a randomized complete block design by Fisher's Protected LSD, and individual tests by chi-square analysis.

The effect of substrate pH on larval survival was studied by placing 100 wandering larvae in small containers with substrates of four pH levels by mixing buffer solutions of pH 4, 6, 8, and 10 with dry vermiculite. Each container was



covered with plastic wrap to maintain the moisture content at approximately 70%. The covering was removed for approximately 20 min each day to allow gas exchange until the flies began to emerge. The number of adult flies were counted and recorded. This test was repeated three times. Data were analyzed as a completely randomized design by Fisher's Protected LSD test.

### Effect of Osmolality

The pupation site selection of larvae for different osmolalities was evaluated with groups of wandering larvae. Osmolality mixtures for the group tests were obtained by mixing  $\text{MgCl}_2$  with water and vermiculite (methods similar to those used by Grodowitz et al. 1987). Osmolality and pH were measured at the beginning and towards the end of the experiment to determine if these measures remained constant throughout the experiment (listed values are means of the first and last measurements). Resulting osmolalities were 111, 254, 403, and 609 mmol/kg. These mixtures were placed in wedge shaped pie sections in a 19 cm diam round container, resulting in vermiculite approximately 2 cm deep. The position of the 4 osmolality mixtures in the pan was randomized. Thirty wandering larvae were introduced into the center of the pan and left undisturbed; pupae in the different sections were recorded 48 h later. Data were analyzed as a randomized complete block design with Fischer's Protected LSD test.

The survival of larvae pupariating in substrates of different osmolality was evaluated. One hundred wandering larvae were placed in four different osmolality

mixtures, which were obtained as described previously. Tests at each osmolality level were replicated three times. The containers were covered with plastic wrap to maintain consistent moisture conditions. The covering was removed periodically to allow gas exchange until the flies began to emerge. After all of the flies emerged, the number of adults was recorded. Data were analyzed as a completely randomized design by Fisher's Protected LSD test.

### Effect of Temperature

The effect of substrate temperature on larval selection of pupation sites was evaluated by establishing a temperature gradient in a concave aluminum trough of 4.1 cm diam and 51 cm long constructed with rectangular aluminum projections at each end. This apparatus was modified from one used by Richard Lee (Dept. Zoology, Miami Univ., Hamilton, OH; personal communication). The projections served as heat exchangers and facilitated the maintenance of a temperature gradient along the length of the trough. The projections were placed in a hot bath and in an ice/water bath. The trough was insulated along its length with foam rubber, while the top was covered with a removable plexiglass cover. Ten holes were drilled on the cover for gas exchange, ease of measuring temperature, and to introduce larvae uniformly along the gradient. Temperature was monitored by inserting mercury thermometers through the holes in the plexiglass. For this study, 25 wandering larvae were introduced via 2 different methods. In the first experiment 25 larvae were placed in the center of the gradient, while in the second

experiment, 25 larvae were introduced uniformly along the gradient. Each experiment was replicated twice.

### Effect of Light

The selection by larvae for different light levels was evaluated. Round sections of Flex-o-film® plastic sheeting (Flex-o-glass, Chicago) were placed over round cake pans (19 cm diam) containing a uniform mixture of vermiculite and water. On the surface of each round plastic disk were glued wedge shaped pie sections, 2 of black construction paper, 2 of black colored cloth, and 2 remained clear plastic placed in an alternating pattern (see Figure 2.); and light levels measured with a light meter under sections were 0, 124, and 710 lux, respectively. Seventy-five wandering larvae were introduced into the center of each pan. Plastic wrap was placed over each pan and the prepared plastic disks were placed directly over the plastic wrap. Each of 3 pans was placed in an environmental chamber (at 27° C) directly under an incandescent 75 W light bulb. A cylinder of black construction paper (22 cm diam by 48 cm) was placed directly over each pan to limit extraneous light. The position of the pupae under the disks was recorded after 48 hours. Data were analyzed in a randomized block design by Fischer's Protected LSD test.

### Effect of Texture

The effect of substrate texture on pupation site selection was evaluated by texture choice tests. Fine grain masonry sand was used to vary the texture of the

media. Sand and water, house fly CSMA (Chemical Specialities Manufacturers Association rearing medium, Ralston Purina Co., St. Louis, MO) media and water, and a mixture of sand, CSMA media and water were the three choices in the texture choice test. These three mixtures were placed in round pans (19 cm diam) in wedge shaped pie sections, with two sand/CSMA mixtures separating the sand-only and CSMA-only mixtures. The moisture contents of these mixtures was measured to be 11.9% (sand-only), 30.1% (sand/CSMA), and 69.9% (CSMA-only). Fifty wandering larvae were introduced into the center of each pan. Three replications were conducted. Pans were covered with plastic wrap and placed in an environmental chamber at 27° C. At the end of the experiment, position of the pupae was recorded. Data were analyzed in a randomized block design by Fischer's LSD test.

#### Effect of Depth

Larval selection for different depths in artificial media was evaluated in containers made from 15.3 cm diam polyvinylchloride drain pipe cut to 35 cm sections. The base end of the pipe was glued to square sections of plate glass. The glass sections were painted black to prevent light from entering the bottom of the columns. CSMA medium, 17.0-17.5 cm deep, was used in all depth experiments with moisture levels ranging from 60-64%. Media compaction levels were achieved by compressing the media with round (15 cm diam) weights. Approximately one-third of the total media needed was placed in the column and weights

were applied for two minutes. This was repeated for the subsequent sections. The weight treatments used were 0, 5, and 20 kg, which resulted in compression levels of 0, 27, and 109 g/cm<sup>2</sup> respectively.

Two hundred wandering larvae were randomly distributed at the top of the column of media. The columns were covered with cloth to prevent larvae from escaping, while allowing for gas exchange in the column. Pupae were counted by removing 1-cm layers and recording number of pupae in each layer. After the first 4 1-cm layers were removed, 2 cm layer sections were removed and analyzed until the bottom of the column was reached. The data were subjected to the SAS General Linear Model (SAS 1987) procedure using the REPEATED statement. Polynomial transformations were made in order to express the data through polynomial relationships. In addition, data were fit to a line; regression coefficients and R<sup>2</sup> values were reported for the log-transformed data.

#### Distribution in Uniform Media

The effect of different moisture contents on the distribution of larvae in uniform medium was evaluated to assess the effects of conspecifics on pupal aggregation, as well as the effects of moisture on pupal aggregation. Treatments consisted of vermiculite with three moisture levels (35, 69, 82 %); each treatment was placed in five 18 cm square pans, 2 cm deep and was replicated five times. The moderate moisture treatment (69%) was also placed in 5 round pans (19 cm diam). In all the square pan treatments, 100 wandering larvae were introduced uniformly



(5 larvae in each cell of a 20-cell grid), while in round pans 50 wandering larvae were introduced uniformly (approximately 12 larvae in each of four pie sections). Larvae in the round and square pans were introduced at the same density level.

The distribution of pupae for each of 15 square and 5 round pan treatments was analyzed using a chi-square analysis, breaking the square pans into 36 cells, and the round pans into 16 cells. These cell sizes were chosen because they seemed to be the largest cell size relevant to the size of the larvae. The chi-square value was used as an index of aggregation to measure the extent of aggregation in the pans. An ANOVA was conducted on the chi-square values of each of the 3 moisture treatments. Chi-square values for the square pans at the moderate moisture level (69%) were also compared to chi-square values for round pans. In a separate analysis, chi-square values for outside cells only versus inside cells only were compared within each treatment via ANOVA.

#### Field Measurements of Pupation Sites

Field measurements of naturally occurring pupation sites were taken at the Beef Research Unit, KSU. Pupae were collected along feedlot pens and feeding troughs with a small sample of the surrounding substrate in several sites in June-August and November in 1986 (6-22, 7-14, 8-15, and 11-8). Pupae were not abundant on any of the collecting dates; every pupae discovered was collected. In addition, samples were taken from a broad range of conditions where there were no pupae. The sum of pupae sites and non-pupae sites may represent an estimate

of the total sites available in the field. Temperature measurements were made in the field with a mercury thermometer prior to the collection in a particular site. Small samples of the surrounding substrate were placed in small glass vials and taken back to the laboratory for further analysis. Wet weights were measured immediately for determination of moisture content. Osmolality and pH measurements were taken within 24 hours of collection. Throughout the experiment, osmolality and pH were not noted to change significantly in this 24 hour period. Site descriptions, substrate type, and stage of pupal development were also recorded. For each parameter measured, a mean, standard deviation, and range were calculated. A discriminate analysis was performed to determine if given a set of environmental conditions one could predict whether sites would be a suitable stable fly pupae site or a non-pupae site. In addition, several pairs of parameters were subjected to correlation analysis.

### Field Tests

A mark-recapture experiment was conducted in the field to determine the minimum and maximum distances larvae wander before pupation. Prepupal larvae were labeled by adding 500 mg of sodium fluorescein (Aldrich Chemical Co., Milwaukee, WI) to 2.4 kg media at least 24 h prior to release. Lab reared larvae were released at the Beef Research Unit in groups of 200 or 300 as soon as larvae began to aggregate in the corners of the rearing pan. Larvae were released in sites from where mature stable fly larvae had been previously collected. Some of these

sites actually contained mature wild stable fly larvae, viable pupae, or pupal exuviae. Larvae were released into 7 different sites on 3 separate days. Larvae were released along the cement of the feedlots and feeding troughs so that larvae would travel along the cement and thus be easy to find after pupating. Pupae were then collected approximately 36 h after larvae were released.

A description of the release site was made and % moisture, temperature, pH, and osmolality were measured. The same data were gathered at the collection sites in addition to number of pupae and the distance the larvae had traveled from the release site. The parameters of the site where larvae were recaptured were compared to the parameters at the release site; both of which were compared to the total sites available in the field. Samples were taken broadly from the feedlot with the intent of including extreme conditions for each parameter.

## RESULTS AND DISCUSSION

More larvae pupated in media with a 71% moisture content when given the choice of 25, 57, and 71% moisture levels ( $P < 0.001$ ;  $df = 3, 6$ ;  $F = 61.52$ ; Table 1). However, larvae did not select 89% over 68% moisture contents in a separate choice test ( $P < 0.05$ ;  $df = 3, 6$ ;  $F = 4.99$ ; Table 1). These results indicate that a relatively narrow range for moisture content was selected by stable fly pupae.

In experiments conducted with two low (25%), one moderate (57%), and one high (71%) moisture levels more larvae pupated in the highest moisture medium

( $P < 0.001$ ;  $df = 3, 6$ ;  $F = 24.61$ ; log-transformed data); however, the distribution of larvae among two high (75%), one moderate (57%), and one low (25%) moisture media was not significantly different ( $P < 0.1$ ;  $df = 3, 6$ ;  $F = 4.09$ ). When larvae were tested individually, the distribution among treatments was not random ( $X^2 = 40.5$ ;  $df = 3$ ;  $P < 0.005$ ), illustrating an effect of the moisture treatments.

Larvae used in all choice experiments were reared in media with ca. 70% moisture. When reared at higher (89%), and moderate (57%) moisture levels, pupae distribution over three moisture levels in two experiments was not random, with the highest number of pupae at 70% moisture in both cases ( $X^2 = 115.686$  and  $14.25$  respectively;  $df = 3$ ;  $P < 0.005$ ). However, larvae reared at low moisture (50%) were distributed randomly when given the same choice ( $X^2 = 2.25$ ;  $df = 3$ ;  $P = \text{n.s.}$ ). Survival to the adult stage of larvae pupating in a high moisture medium (89%) was lower than that of pupae in lower moisture levels of 51 and 68% ( $P = 0.0614$ ;  $df = 2, 7$ ;  $F = 4.27$ ; Table 1). In a separate experiment, survival was unaffected by lower moisture regimes (25, 57, 71 %;  $P = 0.5072$ ;  $df = 2, 7$ ;  $F = 0.75$ ). In the field, larvae were found in moistures ranging from 36-73% moisture level (Table 2). This is in the upper end of the range of total sites available. Total sites in the table is an estimate of what might be the total sites available to the larvae, which was derived by combining parameter measurements of pupae sites and non pupae sites. For moisture content, these data indicate larval selection away from the drier areas in the feedlot. Rasmussen and

Campbell (1981) found stable fly immatures (both larvae and pupae) at moderate moisture levels, at a mean of 52%, with a range of 22.9-65.0%.

Pupal survival in different substrate moisture levels was essentially identical at all moistures between 25 and 71%, yet was significantly lower at 89%. This indicated that at the levels investigated, high moistures were more stressful for pupae than lower (25%) moisture levels. Hoffman (1968) demonstrated that stable fly pupae were highly tolerant to water, and could withstand up to 142 h immersed in water and still emerge, however, no pupae could survive longer periods totally immersed. If more extreme dry conditions had been tested in this study, the results might have shown more significant effects of desiccation, resulting in decreased survival.

Wandering larvae selected a narrow range of temperatures (24 and 28 °C; Fig 1). The distribution of pupae along the temperature gradient was not different whether the larvae were released centrally or uniformly ( $P = \text{n.s.}$ ;  $\text{df} = 1, 17$ ;  $F = 0.06$ ); the number of pupae from both experiments peaked between 24 and 28° C, respectively (Fig 1). Survival was also influenced by temperature. The middle temperature (26 °C) resulted in significantly higher survival (92.1%) than either the lower (21 °C) or higher (32.5 °C) temperatures (86.4%, 84.3%, respectively;  $P = 0.0934$ ;  $\text{df} = 2, 7$ ;  $F = 3.39$ ). Sutherland (1979) reported an optimal range for pupal survival to be from 20 to 30 °C, which corresponds roughly to the range at which larvae pupated along the temperature gradient. Rasmussen and



Campbell (1981) found stable fly immatures in the field at a mean temperature of 7.7 °C, and a range of 21.1-25.3 °C, which closely resembles the results of this study.

Although larvae selected a specific range of temperatures when given a choice, temperature of the substrates in the field tended to reflect the ambient temperature. Although some substrates may be intrinsically higher in temperature, such as decomposing silage, larvae may not be able to choose among different temperatures in the field situation because temperatures may be fairly homogeneous within the area traversed by a wandering larvae. This argument is supported by the data in Table 2 since the measurements of temperatures in the estimate of total sites available in a feedlot (13-29 °C) mirrored the sites in which pupae were present (13-29 °C). This indicates that little or no active selection for different temperatures by the insect is occurring; whereas in the three other parameters measured, the pupal site range was always narrower than the measurements of the total sites available. One notable exception might be those larvae that are overwintering in warm silage (Scholl et al 1981; Hall and Smith 1986).

When given a choice of media with 4 different pH levels (5.2, 6.4, 7.2, 9.3), the greatest number of pupae were recovered at the highest pH, 9.3 ( $P < 0.001$ ;  $df = 3, 9$ ;  $F = 24.45$ ; square-root transformed data; Table 3). Survival to the adult stage at these pH levels was highest at 7.2 and lowest at 9.3, but not different at the two lower pH values ( $P < 0.001$ ;  $df = 3, 8$ ;  $F = 36.62$ ). When tested individu-

ally, distribution of stable fly pupae was not random, with most pupae at pH 9.3 ( $X^2 = 13.76$ ;  $df = 2$ ;  $P < 0.01$ ). When larvae were subjected to the three lower pH levels, they pupated evenly among the treatment when tested in groups ( $P = \text{n.s.}$ ;  $df = 2, 6$ ;  $F = 3.48$ ), or individually ( $X^2 = 1.52$ ;  $df = 2$ ;  $P = \text{n.s.}$ ). The range of pH where pupae were found in the field was 6.4-8.3 (Table 2). Pupae were found in the upper range of available sites, ignoring the low pH substrates. Rasmussen and Campbell (1981) also found stable fly immatures at pH 7.7, with a range of 7.1-8.2.

The number of pupae was highest at 111 mmol/kg medium ( $P < 0.001$ ;  $df = 3, 9$ ;  $F = 16.84$ ; Table 4). Numbers of pupae were lower at 254, and 403 mmol/kg, and lower still at 609 mmol/kg. Pupal survival in different osmolality treatments was also significantly different ( $P < 0.01$ ;  $df = 2, 6$ ;  $F = 18.58$ ). Mean survival to the pupal stage was highest at the two higher osmolality levels (Table 4). Similar to pH, insects were found in the lower end of the range of total sites available (Table 2).

Survival in substrates at different pH was a more complex story. Survival appeared to be lowest (19.0%) at the high pH (9.3), which was significantly selected by larvae over all three lower pH levels. I believe this datum point (19.3%) is anomalous and does not reflect the true survival of pupae at high pH. The rearing media often reaches 9.0 or higher as the insects begin to pupate (Broce, Kansas State Univ., personal communication). Also, pH in the field

(according to my measurements) never reached higher than 8.3, and larvae may never encounter the high pH levels used in the choice tests. In fact, several authors have found indirect correlations between face fly larvae mortality and low pH levels (<6.0) in feces of cattle fed high grain diets (D'Amato et al. 1980, Meyer et al. 1980, Grodowitz et al. 1987). D'Amato et al. (1980) suggested that face fly larvae were intolerant of low pH levels.

The selection of both high pH and low osmolality was not surprising. This study found that pH and osmolality were negatively correlated (Pearson correlation coefficient = -0.57;  $P < 0.0001$ ). Haas (1986) and Grodowitz et al. 1987 both reported a negative correlation between pH and osmolality ( $r = -0.78, -0.98$ , respectively). In addition, moisture and temperature were slightly positively correlated (Pearson coefficient = 0.27067;  $P = 0.0095$ ), as was pH and temperature (Pearson coefficient = 0.25508;  $P = 0.0224$ ).

Insects were found to pupate in the dark, given a choice of light levels: 0, 124, and 710 lux ( $P < 0.01$ ;  $df = 2, 4$ ;  $F = 18.34$ ), yet there were no differences in survival at the three light levels ( $P = 0.19$ ;  $df = 2, 7$ ;  $F = 0.8301$ ). This may be due to the fact that pupating in the dark may be a proximate mechanism for consequences beyond the physical effects of light on the pupae itself, consequences not addressed by laboratory assays. Escaping light, larvae tend to pupate under the surface of its substrate, where they are less subject to desiccation and less vulnerable to predators and parasites. This idea is analogous to the findings

of Rizki and Davis (1953) indicating that response to light played a role in niche differentiation, with a concomitant decrease in interspecies competition, however light itself had no direct effect on pupal survival.

Larval selection of sand over CSMA media and a mixture of sand and media ( $P < 0.01$ ;  $df = 2, 4$ ;  $F = 60.21$ ) suggested that texture was an important variable in site selection. Due to differences in water absorption capabilities of the different substrate types, effects of moisture and texture were confounded by the fact that sand had a 13% moisture content while the CSMA media contained 70% moisture. Sand was still selected over CSMA media, even though larvae normally select moisture levels around 70%. Sand has commonly been used as a stable fly pupae trap, both in the laboratory and in the field. This is an effective technique, however its effectiveness does not appear to be simply due to a tendency to pupate at lower moisture levels as it has been demonstrated herein that larvae have selected moisture contents of up to 71% in different substrates.

All choice tests were conducted with groups of larvae, which created an intrinsic problem in the choice tests since larvae tended to aggregate. In group tests the effect of the parameter was confounded with the effect of conspecifics on aggregation. Moisture and pH choice tests were also conducted with individual larvae, producing essentially the same results as with groups of larvae. The 'group effect' was the tendency of larvae to aggregate in the choice test. Tests were conducted with two moderate sections and due to the group effect, most

pupae were found in one moderate section while significantly fewer pupae were in the other moderate section. This phenomenon lead to high variances, making it more difficult to demonstrate differences between the treatments. This is evident in the first section of Table 1, where large differences between means appear not to differ. Yet it is quite clear that insects selected media with 68% over 50 and 89% moisture.

The group effect was further analyzed by examining the distribution of pupae in uniform media (no choice test). The chi-square value was used as an index of aggregation (see Table 5). These indices were significant ( $p < 0.01$ ) for every test, indicating that the distribution of pupae was not random. Zvereva (1986) found similar results for house fly larvae. House fly larvae show a non-random distribution in uniform media. In the present study with stable flies, the extent of aggregation was significantly higher in the moderate moisture treatment (69%) compared to the high (82%) and low (35%) moisture treatments ( $P < 0.0001$ ;  $df = 2, 11$ ;  $F = 21.58$ ; Table 6). This phenomenon may indicate a preference for the moderate moisture level, as was observed in the choice tests. One underlying assumption was that under suboptimal conditions, wandering larvae continue to search for better conditions for longer periods of time. This assumption was tested in other experiments (see Chapter 2). When testing the extent of aggregation in the outside cells of the pan only, the same results were found; the extent of aggregation was higher at moderate moisture levels ( $P < 0.001$ ;  $df = 2, 11$ ;



$F = 19.41$ ) (Table 7). However, the extent of aggregation was not different among moisture levels when testing the inner cells only ( $P = \text{n.s.}$ ;  $df = 2, 11$ ;  $F = 1.7$ ). The outer cells of the pan must be responsible for the differences observed in the entire pan (Table 6).

Distribution of pupae in the peripheral or outer cells was not significantly different from that in the inner cells in low (35%) moisture treatment ( $P = \text{n.s.}$ ;  $df = 1, 8$ ;  $F = 2.74$ ); however the index of aggregation was higher for outer cells in both the moderate (69%) moisture level ( $P < 0.001$ ;  $df = 1, 6$ ;  $F = 643.55$ ; log transformed data) and in the high (82%) moisture treatments ( $P < 0.001$ ;  $df = 1, 8$ ;  $F = 50.06$ ; square-root transformed data; Table 7). There was no difference between extent of aggregation in round pans (with 9 cells) than in square pans (with 36 cells;  $P < 0.238$ ;  $df = 2, 11$ ;  $F = 2.16$ ).

The experiment with square pans demonstrated the 'edge effect,' the tendency of larvae to pupate along an edge or attached to some vertical substrate. In the outside cells, there were edges and corners. The extent of aggregation may have been due to an increased probability of encountering other larvae or pupae because movements are no longer random; insects tend to follow the edges of the pan. The differences may in fact reflect the artificial containers of the treatment, although cement edges in feedlots may function in a similar manner.

Zvereva (1986) found that house fly larvae selected media which had previously contained house fly larvae over media which had not contained any larvae.

House fly larvae were also attracted to a water extract of media which had contained larvae. Zvereva (1986) claimed that the larvae may have been attracted to by-products of metabolism, or to bacteria remnant from the previous larvae.

Experiments in the present study on distribution of pupae in uniform media indicated that contact of other larvae and pupae may have caused pupal aggregations, although alternative explanations (eg. attraction to bacteria or by-products of larval metabolism) cannot be ruled out.

Working under the assumption that insects tend to maintain optimum population densities, Zvereva (1986) examined the effect of group size on survival and pupal weight in house flies. Individual larvae had lower survival and decreased pupal weights than larvae in groups of 5 and 100 larvae/g medium. When placed under two sets of unfavorable conditions, low pH (5.6), and media with high moisture levels (85%), these differences were significantly magnified. This was contradictory to the results of the present study assessing stable fly pupal distribution in uniform media. For *Stomoxys calcitrans*, the extent of pupal aggregation under high moisture conditions (89%) was lower than aggregation at moderate levels (68%; Table 6). Zvereva (1986) speculated that groups of larvae under less favorable conditions could manipulate their microclimate to make it more favorable, whereas individuals could not affect the microclimate; yet *S. calcitrans* aggregated in moderate conditions and not in extreme conditions. Takahashi et al. 1966 demonstrated that house fly larvae could change (increase) the pH of the

immediate environment 1-2 pH units. They suggested that the increase in pH was due to either the ammonia excreted by the larvae, microorganism activity, or the physical mixing from the larvae themselves (Takahashi et al. 1966). Zvereva (1986) claimed that this explained the increase in the higher survival at low pH levels. In addition, Zvereva (1986) demonstrated that groups of larvae (5 and 100/100 g medium) outcompeted the fungus in the medium, and Zvereva (1986) suggested that the insects might be excreting a fungicide. However, a more feasible explanation is that the movement (mixing of the medium) by the mass of larvae facilitated prevention of the spread of the fungus throughout the medium, as suggested by Lussenhop et al. 1980. The fungus may also have been prevented from spreading by a grazing effect of the larvae. At low larval densities, mold and fungus tie up nutrients in the media by presenting physical barriers (i.e. mycelium of fungus). High larval densities disrupt mycelium formation as a barrier to larval movement.

The discrepancy between Zvereva's (1986) result of increased aggregation under unfavorable conditions, and my result of decreased aggregation in unfavorable conditions, may be due to differences between larval and pre-pupal behavior, or between house fly larvae and stable fly larvae. As stated previously, there are many proximate and ultimate causes for larval aggregation. Pupae, however, do not feed, and the nutritional and bacterial differences in the media may be irrelevant. Yet, too little is known about behavior of wandering stable fly larvae

to ignore the influences of bacteria and other physiological factors affecting pupation in a group. The adverse effects of pupating in a group would be similar to those affecting larval aggregations, such as effects of parasites, predators, and the spreading of diseases in the population. The discrepancy between Zvereva's (1986) study and this study may be that the adverse effects, particularly of parasitism or aggregation may be significantly more severe for pupae than for larvae. In addition, both studies were conducted in the laboratory under very restricted, artificial conditions. Neither of the results may be relevant to what larvae and pre-pupae do in the field.

In this study very few pupae were found in the field in aggregations. Parr (1962) claimed to have discovered "seething masses of *Stomoxys* larvae and also pupae." Consistent with my findings, Gilbertson and Campbell (1986) reported that stable fly larvae and pupae were never found in aggregations. The reason for not finding aggregations of pupae may be due to the differences in larval and pupal behavior as discussed previously. Although the results of the no choice tests in uniform media may be somewhat artificial, there were many cement structures providing vertical edges in feedlots and dairy farms. Many of these structures discussed previously have been cited as important immature stable fly breeding sites in an evaluation of cattle feedlot systems by Gilbertson and Campbell (1986).

Depth experiments were also conducted to assess the role of depth and den-

sity of substrate in pupation site selection. Substrate density levels were chosen to simulate conditions encountered by larvae and pupae in the field. The highest density tested was used to simulate very dense substrates (i.e. compacted soil by continuous cattle movement in feedlots). The lower densities may not simulate soil densities, but do reflect the loose silage, manure, soil, etc. that may accumulate in areas inaccessible to cattle (eg. along cement feeding troughs and drinking troughs). At the most dense compression level ( $109 \text{ g/cm}^2$ ), pupae were not found below 4 cm. Only at the lowest treatment ( $0 \text{ g/cm}^2$ ) did insects pupate at the bottom of the column (12 cm). However, in the laboratory depth experiment, the majority of pupae (94.9%) were found above 4 cm, while 82.0% of the pupae were found above 2 cm. Even in loose media most pupae were found in the top 2-4 cm, consistent with where most pupae were found in the field.

There were significant differences in the distribution of pupae in the media columns of differing densities (Fig. 3). Polynomial transformations were made in order to express the data through polynomial relationships. First through seventh degree polynomial contrasts were generated by the General Linear Model Procedure (GLM; SAS 1987). The GLM procedure found overall significant differences between treatments (0, 27, and  $109 \text{ g/cm}^2$ ;  $P < 0.002$ ;  $df = 2, 6$ ;  $F = 20.87$ ). The Analysis of Variance of polynomial orders 1-7 was tested for both mean and treatment effects (Table 8). Mean effects represent overall trends in the data set, while treatment effects indicate that the nature of the  $n^{\text{th}}$  poly-



mial is different depending upon the treatment. There is a significant treatment effect for the first order polynomial as well. This means that the linear trends for each of the three treatments are significantly different. The entire data set has both a significant linear trend, and a slightly less significant quadratic trend, due to the first and second order polynomial mean effects. Lines were also fit to each of the three levels of compaction. Regression coefficients for these lines are given in Table 9. The slopes and intercepts are very similar for the two lower levels, but the slope for the most dense treatment is somewhat steeper (Fig 3). This supports the idea that most insect pupate relatively near the surface and are less likely to be found significant distances below the surface as the substrate becomes more compacted.

Lab-reared larvae were released in the field to determine how far wandering larvae would travel and if these larvae would select better conditions from sites available in the immediate vicinity. Conditions were considered better if they produced higher survival or pupal weights in the laboratory study. However, in the field there may be several other factors important for survival besides the five parameters studied in the laboratory. In light of the previous discussion, wandering larvae were placed along a cement edge of the holding pens or water troughs, to facilitate their recapture. Larvae were removed from a standard rearing pan; each pan contained a wide variation of physiological stages, from second instar larvae to tanned pupae. In a pan of 600 larvae, it has been demonstrated that it

takes in excess of 70 hours for every larvae in the pan to complete pupariation, while the wandering phase length was ca. 13 h, and the process of pupariation lasted ca. 6 h. (see chapter 2). Since there was wide variation in physiological stages in any of the rearing populations, those larvae released in the field that did not move were probably at the end of the wandering phase.

The mean distance traveled was 40.4 cm. The relevant information from the mark-recapture study was the maximum distance traveled (911.9 cm). This is a conservative estimate of distance traveled due to the relatively low number of pupae recaptured (17.5%). Given this long distance, larvae appear to have the ability (or sufficient time) to 'search' relatively long distances to find more suitable conditions for successful pupation. However, there was no evident relocation to conditions such as those selected in the laboratory choice tests when comparing release sites to recapture sites. Conditions which were 'better' than the release sites may not always have been available in the near vicinity. The time spent wandering may or may not be sufficient to allow the larvae to choose an optimal site from among spatially and temporally limited sites available. Larvae could travel up to ca. 9 m during the wandering phase, however, this phase is largely hormonally controlled, allowing for some manipulation of the timing of pupariation (see chapter 2). Thus, at any one point in time, suitable conditions may not be present in space or in time. Larvae do not 'search' for better conditions, but if they are in unfavorable conditions, and they continue to crawl, this will bring

them out of their immediate surroundings. Larval perception is limited to its immediate surroundings, and can certainly not predict what other conditions are nearby. There is likely a window of time in which the larva can accept a site, and this may be influenced by physiological state and external stimuli (see Chapter 2). There may be a critical minimum distance the larva must move before it can accept any site. More work is needed at the individual insect level to determine mechanisms of movement, and behavior patterns during the wandering phase. Finally, the process of removing wandering larvae from one habitat (the laboratory rearing media) and placing them into the feedlot may have disturbed the larvae into exhibiting abnormal behavior.

Alternatively, in the field measurements of pupae and non pupae sites, the range of parameters of pupal sites is narrower than the total range of parameters available in the field (except temperature, Table 2). According to this information, larvae are selecting to some degree certain moisture, pH, and osmolality levels. Although, selection of sites for larvae and pupae may be limited by oviposition selection by adult female stable flies.

The selected ranges of four parameters examined in the laboratory and measurements of sites where pupae were collected in the field are compared in Table 2. Included in this table are measurements of non-pupae sites in the field. The sum of pupae sites and non-pupae sites were used to represent my estimate of what might be the total available sites in the field. These sites were compared to the

actual pupal sites. A discriminate analysis was conducted using four parameters, moisture, temperature, pH, and osmolality to categorize stable fly pupae sites and non pupae sites (Table 10). Using the same data to assess the model, the percentage of observations from pupae sites classified correctly as pupae sites was 88.89%, while 83.33% of the non pupae sites were correctly classified into non pupae sites. The coefficients used by the model indicate their relative importance to the predictive power of the model (Table 10). Accordingly, pH appears to be the most important variable in this model.

As was stated previously, several authors have demonstrated that low levels of pH may increase mortality of face fly larvae (D'Amato et al. 1980, Grodowitz et al. 1987). The mean of non pupae sites is lower than the mean for pupae sites (Table 10), indicating that wandering larvae are avoiding the low pH levels in the field. These authors worked with face flies, which may have very different nutritional needs due to their intake of minerals for the formation of the hard pupal cuticle of face flies (Grodowitz and Broce 1983). This experiment ranked pH as the most important environmental variable of the parameters in this study that was influencing site selection. However, other studies on suitable stable fly sites have been unable to correlate larval breeding sites with pH (Rasmussen and Campbell 1981, Gilbertson and Campbell 1986), suggesting the incomplete nature of using only environmental parameters to predict larval or pupal presence.

This study gave inconclusive results concerning the ability of wandering lar-

vae to relocate under 'better' conditions. Pupation site selection is a complex behavioral process which involves responses to a variety of environmental and physical parameters. The fact that larvae do not appear to relocate under near optimum conditions for each parameter has two major implications. The first is that there may be a hierarchical response to environmental parameters, where one or two parameters are evaluated at a higher level, or are of more importance than other parameters. A speculative hierarchical order of parameters may be inferred from the combination of laboratory choice test, survival test, field ranges for each parameter, and the discriminant analysis. Temperature does not appear to be of much importance in the selection process. As was stated previously, the substrate temperature generally reflects ambient temperatures, resulting in very small fluctuations of temperatures in localized areas at one point in time. The observed differences in temperature that one does see are due to differences between shaded and non shaded areas in the feedlot. In addition, the temperature range of pupal sites in the field reflected the exact range of total sites available in the field. Thus temperature may have less importance in determining site selection, although temperature did affect survival of the insect.

Light, on the other hand, had no effect on survival. Although insects pupariated in complete darkness, this negative phototropism may affect vertical, rather than horizontal movement of larvae. Light does cause the larvae to burrow into the soil, allowing them to escape desiccation from the sun. Although this is



critical to survival, there seems to be more diverse conditions horizontally than vertically. Thus light may be of reduced importance in horizontal pupation site selection. It is difficult to rank the remaining parameters, moisture, pH, and osmolality in any order in terms of their importance in site selection on the information available in this study. Although due to the results of the discriminant analysis, pH may be of the highest priority due to its effects on mortality at low levels. However, there was no other evidence in laboratory tests or field measurements that indicated that pH would be of higher significance. This type of analysis is very reductionist, and does not allow for interactions between parameters, and alternative selection pressures on site selection. For example, in Chapter 2 there was a highly significant interaction between temperature and moisture in their effect on survival of stable fly pupae to the adult stage. Only at both high temperatures and high moisture levels was there a highly significant decrease in survival.

For this type of hierarchical ranking of parameters in their order of importance, it is a useful exercise to imagine that insects would pupariate at an optimal range for each parameter. However, in reality, it is not expected that insects would choose pupation sites based simply on optimal survival as determined in laboratory assays. The response to environmental parameters should not be considered in isolation of the rest of the biology of stable fly larvae (Gould and Lewontin 1979). Biotic factors (not measured in this study) may prove to be more

important than environmental conditions of pupation sites to the eventual success of the organism. The selection of pupal sites is definitely not a single trait on which selection may act simply to ensure maximum survival. But it is inextricably linked to the selection of all other traits besides site selection.

Day to day weather changes will alter environmental parameters in the field on a regular basis. Certain weather conditions have been known to affect stable fly populations directly. Fuller (1913) reported outbreaks of adult stable flies directly following floods in South Africa. Hall et al. 1982, more recently, linked high stable fly adult populations to heavy rainfall. Also, over the 5-6 days as pupae (Mitzmain 1913) the immediate conditions, particularly temperature, may change significantly. This confounds the comparison between pupation site selection and survival under certain conditions since the conditions at the time of pupation may change during the entire pupal stage.

This study does not examine all possible parameters involved in stable fly pupation site selection. The effect of texture, or substrate type is not fully understood, and may play a larger role in site selection than was previously assumed. Although the insect is no longer feeding, it is still in association with a multitude of microorganisms. The fauna of microorganisms is an essential component to suitable larval breeding media (Glaser 1924), however, their relation to pupal development, behavior, and site selection is virtually unknown. Lussenhop et al. 1980 reported that *Neomyia cornicina* larvae increased bacterial turnover in the

laboratory, implying a microbial interaction. They also found *N. cornicina* larvae associated with high bacterial densities in the field. Zvereva (1986) found that house fly larvae were attracted to media that had previously contained house fly larvae. These studies demonstrated that other factors besides the 5 parameters used in this study have been important in selection of larval and possibly pupal sites. Alternative selection pressures on site selection should also be investigated.

Major selection pressures that have not been addressed in this study are the effects of predation, parasitism, and interspecific competition. Axtell (1970) surveyed filth fly immature predators, and has found that they accounted for a large, yet undetermined amount of mortality among dipteran immatures. Parasites also have a relatively large but unquantified impact on survival. Smith et al. 1985 estimated that between 4 and 21% of seasonal mortality of stable flies was attributed to parasites. Rizki and Davis (1953) suggested that light may be a determinant of the ecological niche of two species of *Drosophila*, resulting in decreased interspecific competition. It is too difficult to assess whether stable fly pupation sites are limiting, hence this factor cannot simply be ruled out. Avoiding interspecific competition, parasites, and predators may be crucial factors in the selection of pupation sites.

No model involving only environmental parameters will sufficiently describe the complex process of pupation site selection. Although a discriminant analysis was conducted, it was not able to fully predict where one might find stable fly

pupae. Although the model predicted correctly 89 and 83% of the pupae and non pupae sites, respectively, the model was self-verified, and in reality it may only be able to predict 50 to 60% of pupal sites correctly. However, due to the importance of other factors, such as structure of the feedlot, transient effects of weather on the parameters studied, interactions between parameters, predation and parasitism selection pressures, a more complete model would be needed.

Because the five parameters studied did not provide a fully predictive model, and because parameters change over the pupal stage it may be useful to look for other means of evaluations to control stable fly populations. Of the parameters studied, pH was the most important, because of its strong impact on the discriminant analysis model. This information may be applied to a control strategy whereby only sites with relatively high pH are eliminated. However, this single method will not be able to control populations in and of itself. It is also not known to what extent pH (or any of the parameters tested) changes over the pupal stage. Pupae tend to aggregate near specific features of a feedlot, hence categorizing the feedlot into structural components (Meyer and Peterson 1983; Gilbertson and Campbell 1986) and targeting these areas for control may in fact be more useful in controlling these insects than predicting sites based on environmental parameters. However, systematic sanitation of only optimal sites may be effective. In addition, this study gives information on pupal sites specifically, which may result in more effective control by natural enemies as parasitoids can be released in

optimal pupal sites only.

The consequences of choosing a less than optimal pupation site may be relatively mild. Survival differences at suboptimal parameter levels were significant, but not great (70-80% compared to 80-90% for more optimal levels). Thus, environmental parameters may provide a gradual selection pressure on site selection. Severe weather conditions such as drought and flood are transient conditions and may have a relatively greater influence on survival. Preferences exhibited by stable fly pupae may reflect the more dramatic bottleneck selection pressures of extreme weather conditions than the gradual selection pressures exerted from environmental parameters. This would account for the discrepancies between ranges selected in the choice tests, and survival test results with each parameter. The interactions between parameters, and the combination of different types of selection pressures would account for the discrepancy between laboratory choice tests and measurements of pupae sites in the field.



Table 1. Number of stable fly larvae pupariation (out of 30) and percent survival in media with different moisture contents.

Percent Moisture	Mean Number Pupae / Section*	Mean Percent Survival*
<b>51</b>	<b>0.33 a</b>	<b>89.5 a</b>
<b>68</b>	<b>11.33 b</b>	<b>88.1 a</b>
<b>68</b>	<b>17.33 b</b>	<b>-</b>
<b>89</b>	<b>1.00 a</b>	<b>77.0 b</b>
<b>25</b>	<b>1.00 a</b>	<b>89.7 a</b>
<b>57</b>	<b>1.67 a</b>	<b>86.7 a</b>
<b>57</b>	<b>1.33 a</b>	<b>-</b>
<b>71</b>	<b>25.67 b</b>	<b>86.3 a</b>
<b>25</b>	<b>0.00 a</b>	
<b>25</b>	<b>1.67 a</b>	
<b>57</b>	<b>3.00 a</b>	
<b>71</b>	<b>23.33 b</b>	
<b>25</b>	<b>0.33**</b>	
<b>57</b>	<b>2.67</b>	
<b>71</b>	<b>8.00</b>	
<b>71</b>	<b>17.00</b>	

\*Means in columns followed by the same letter are not significantly different as  $P < 0.05$ .

Fisher's Protected L. S. D.

\*\*F test from ANOVA not significant.

Table 2. Summary of laboratory choice tests for stable fly pupae of five parameters, and measurements of pupal sites in the field.

Parameters	Laboratory Experiments	Field measurements	
		Pupae Sites $\bar{x} \pm \text{S.D.}$ (Range)	Total Sites $\bar{x} \pm \text{S.D.}$ (Range)
Temperature (°C)	25-28	24.8 $\pm$ 5.7 (13-29)	22.1 $\pm$ 6.8 (13-29)
% Moisture	68-74	61.0 $\pm$ 10.0 (36-73)	53.9 $\pm$ 19.7 (11-73)
pH	9.0-9.3	7.7 $\pm$ 0.5 (6.4-8.3)	6.2 $\pm$ 1.2 (4.5-8.3)
Osmolality	100-200	171 $\pm$ 73 (91-369)	372 $\pm$ 187 (91-731)

Table 3. pH choice test and survival in media of different pH of stable fly pupae.

pH	Mean Number Pupae / Section*	Mean Percent Survival*
5.2	0.5 a	73.0 a
6.4	2.0 b	71.0 a
7.2	4.8 b c	93.3 b
9.3	17.8 c	19.0 c

\*Means in columns followed by the same letter  
are not significantly different as  $P < 0.05$ .  
Fisher's Protected L. S. D.

Table 4. Osmolality choice test and survival in media of different osmolality levels of stable fly pupae.

Osmolality (mmol/kg)	Mean Number Pupae / Section*	Mean Percent Survival*
111	18.0 a	91.3 a
254	4.5 b	92.3 a
403	7.3 b	80.0 b
609	0.5 c	74.3 b

\*Means in columns followed by the same letter  
are not significantly different as  $P < 0.05$ .  
Fisher's Protected L. S. D.

Table 5. Chi-square values for pupal distributions in uniform media.

Treatment	Pan	Aggregation Index Chi-sqaure*
Low Moisture 35%	Square	228.5 324.7 109.8 159.4 332.1
Moderate Moisture 69%	Square	686.4 1110.3 1051.0 805.2
High Moisture 82%	Square	699.3 326.2 371.5 263.0 444.5
Moderate Moisture 69%	Round	95.2 117.3 74.2 133.7 141.1

\* All chi-square values are significant at  $P < 0.05$ ;  $df = 35$ .



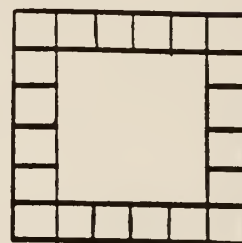
Table 6. Aggregation indices (represented by chi-square values) for stable fly pupae in pans of uniform media with different moisture contents. Means represent five chi-square tests.

% Moisture	Mean of $\chi^2$
35	231 a
69	913 b
82	421 a

\*Means in columns followed by the same letter are not significantly different as  $P < 0.01$ .  
Fisher's Protected L. S. D.

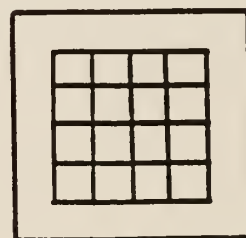
Table 7. Aggregation indices (represented by chi-square values) for stable fly pupae in pans of uniform media with different moisture contents. Means represent five chi-square tests. The ANOVA tested for differences in the aggregation indices of outside cells only, and inside cells only.

OUTSIDE CELLS ONLY	
% Moisture	Mean of $\chi^2$
35	120 a
69	472 b
82	221 a



Pan

INSIDE CELLS ONLY	
% Moisture	Mean of $\chi^2$
35	59.4 a
69	11.3 a
82	21.7 a



Pan

\*Means in columns followed by the same letter are not significantly different as  $P < 0.01$ .  
Fisher's Protected L. S. D.

Table 8. Partial ANOVA table for depth experiment depicting mean and treatment effects for polynomial orders 1-7. three treatments represent three different density levels of media, while levels represent depth of the media.

Polynomial Degree	Source	df	Sum of Squares	Mean Square	<i>F</i> value	<i>P</i> value
1	Mean	1	22.3564	22.3564	401.18	0.0001
1	Treatment	2	1.3445	0.6722	12.06	0.0079
1	Error	6	0.3344	0.0557		
2	Mean	1	4.7975	4.7975	116.82	0.0001
2	Treatment	2	0.6038	0.30188	7.35	0.0243
2	Error	6	0.2464	0.0411		
3	Mean	1	0.4853	0.4853	26.08	0.0022
3	Treatment	2	0.0044	0.0022	0.12	0.8904
3	Error	6	0.1116	0.0186		
4	Mean	1	0.0117	0.0117	0.44	0.5333
4	Treatment	2	0.1813	0.0907	3.37	0.1042
4	Error	6	0.1612	0.0269		
5	Mean	1	0.0197	0.0197	1.06	0.3432
5	Treatment	2	0.0347	0.0174	0.93	0.4437
5	Error	6	0.1116	0.0186		
6	Mean	1	0.0198	0.0198	2.96	0.1361
6	Treatment	2	0.1446	0.0723	10.80	0.0103
6	Error	6	0.0402	0.0067		
7	Mean	1	0.4205	0.4205	117.33	0.0001
7	Treatment	2	0.0407	0.0204	5.68	0.0413
7	Error	6	0.0215	0.0036		

Table 9. Regression coefficients for lines fit to number of pupae plotted against depth in a column of media.

Compression Level (g/cm <sup>2</sup> )	Slope	Intercept	R <sup>2</sup>
0	<b>-0.18330289</b>	<b>1.7281583</b>	<b>0.7325</b>
27	<b>-0.1695596</b>	<b>1.74162861</b>	<b>0.8169</b>
109	<b>-0.09898782</b>	<b>1.60792998</b>	<b>0.6299</b>

Table 10. Means of parameters for both stable fly pupae sites and non sites in the field, including the coefficients for the discriminant analysis model for standardized parameter measurements.

Parameter	Sites*	Non Sites**	Coefficient
Temperature	24.65	22.46	0.281
Moisture	60.77	57.56	1.022
pH	7.86	6.64	14.1482
Osmolality	172.40	295.24	0.5505

\* n = 27

\*\* n = 36



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Figure 1. Distribution of stable fly pupae over a temperature gradient. Wandering larvae were released uniformly along the gradient in test 1., and released in the center of the gradient in test 2.

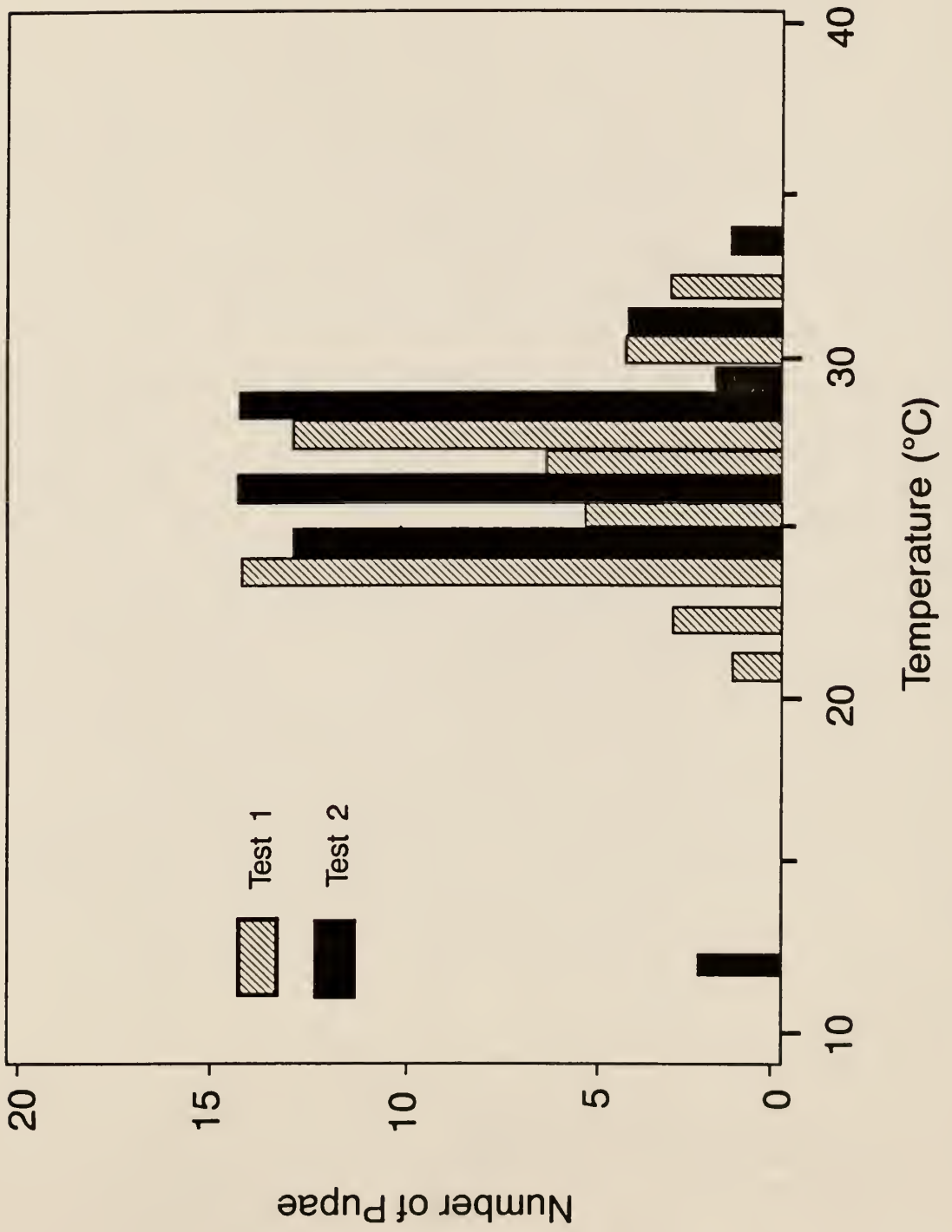




Figure 2. Design for light choice test.

# Light Choice Design

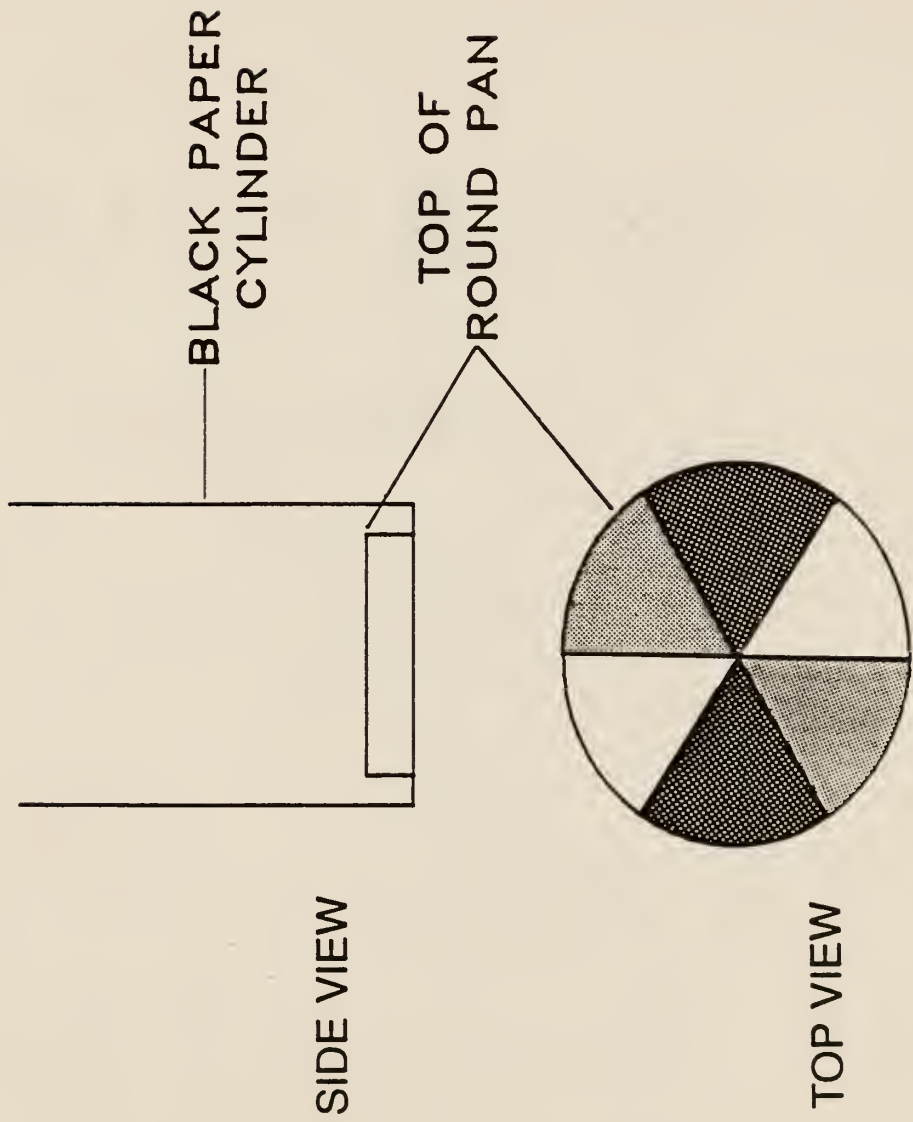
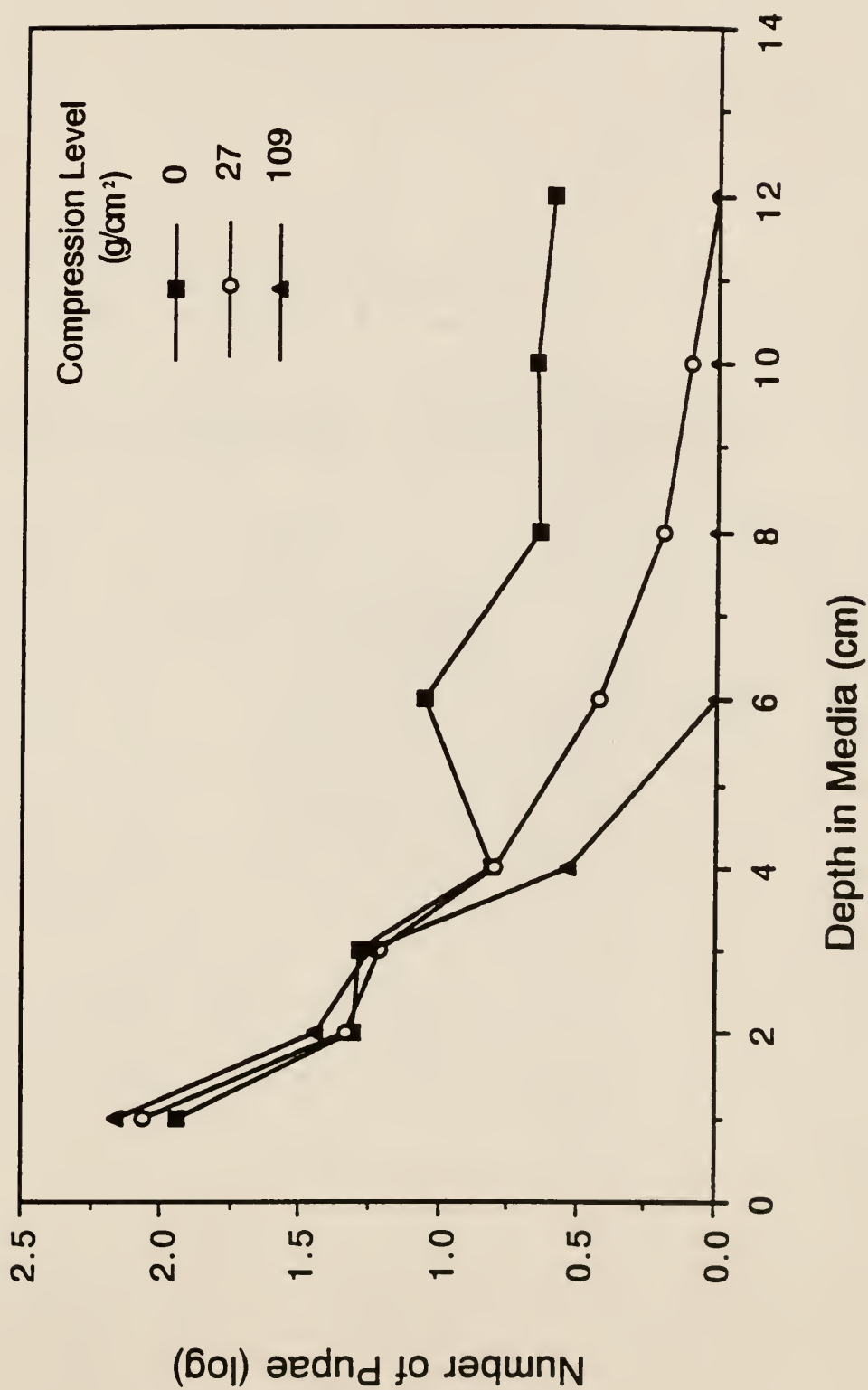


Figure 3. Distribution of stable fly pupae in media columns of different density.



PAPER 2: Environmental Components of Wandering Behavior of the Stable Fly,  
*Stomoxys calcitrans*, (Diptera: Muscidae)

To be submitted to the Annals of the  
Entomological Society of America



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ABSTRACT. The mean wandering phase length for mature larvae of the stable fly, *Stomoxys calcitrans*, was estimated to be  $10.6 \pm 3.5$  h. There was a high degree of variation in the wandering length of a population of 600 larvae reared in the laboratory. In a factorial experiment in which wandering larvae were allowed to pupate in media with three levels each of moisture, temperature and light, the mean time to 50% pupariation was delayed at high, 84%, moisture levels relative to 17 and 67% moisture. The rate of pupariation was also slowest at 84% moisture, but this effect was only seen at the moderate light level (124 lux), and not at 0 or 710 lux. Pupae in media with higher moisture contents had significantly higher fresh weights. Pupae in media with 67 and 84% moisture contents had significantly higher pupal moisture contents than at the low moisture (17%). There were no effects of any of the three parameters on dry weight of pupae. Percent emergence was higher at 17 and 67 than at 84%. There was a significant interaction between the effects of moisture and temperature on survival; only at a high moisture of 89%, and a high temperature of 32° C was there a significant decrease in survival. Density was demonstrated to have no effect on mean time to 50% pupariation or rate of pupariation when comparing groups of 50, 100, and 200 larvae per 200 ml wet vermiculite to insects held individually for pupariation. Puparium formation appears to be random under a 16:8 light:dark cycle, showing no apparent circadian rhythm.

KEY WORDS Insecta, stable fly, pupariation, wandering, circadian

## INTRODUCTION

HOLOMETABOLOUS LARVAE, towards the end of the last instar, undergo hormonal changes which are accompanied by behavioral changes (Truman and Riddiford 1974). In Lepidoptera, the termination of the feeding stage is followed by increased locomotor activity, purging of the gut contents, and negative phototaxis (Truman and Riddiford 1974). These events characterize larval wandering behavior. Some or all of these physiological and behavioral changes occur in several dipteran larvae (Zdarek 1985).

Several studies demonstrate tremendous plasticity in the initiation of puparium formation. A delay in pupariation under conditions of high moisture occurs in *Sarcophaga bullata* (Zdarek and Fraenkel 1972), *Aldrichina grahami*, *Calliphora lata*, *S. peregrina* (Ohtaki 1966), and *S. argyrostoma* (Saunders 1975). Pupariation in *S. peregrina* was delayed over 100 h when the larvae were in wet rearing conditions (Ohtaki 1966).

Wandering behavior is presumed to be induced by ecdysteroids (Fraenkel 1934). The release of prothoracicotropic hormone from the brain (initially stimulated by an earlier release of ecdysone), is thought to be the gated event controlling larvae wandering and puparium formation (Truman and Riddiford 1974, Richard et al. 1986, Berreur et al. 1979b). Hormonal control of wandering behavior may impose limits on the insects' ability to affect the length of wandering, and on the ability of the larvae to choose a suitable site in that window of

time.

The timing of wandering and pupariation are important in finding suitable pupation sites. Richard et al. 1986 found that in *S. argyrostoma*, the initiation of larval wandering occurred as a gated circadian rhythm; in contrast, formation of the puparia was not rhythmic. These authors discussed possible advantages to gated nocturnal wandering behavior, such as avoidance of desiccation, or diurnal predators.

The wandering behavior of an insect is an important phase in that the insect is searching for a suitable pupation site. Once the insect has pupariated, it remains inactive at this site until the adult emerges. The pupal stage is subject to environmental conditions such as desiccation and biotic factors such as predation and parasitism. Hence the consequences of the process of wandering behavior and pupariation are obvious. Much is known concerning the wandering behavior of *Sarcophaga* (Fraenkel 1934, Ohtaki 1966, Zdarek and Fraenkel 1970, 1972, Zdarek 1985, Bradley and Saunders 1986, Richard et al. 1986), comparatively little information is known for the stable fly, *Stomoxys calcitrans* L. (Hoffman 1968). In chapter 1, the pupation site selection for different levels of temperature, moisture, and light were assessed for wandering stable fly larvae. The present study was undertaken to investigate the wandering phase itself. This study examined how the factors (temperature, moisture, light, and density) affected the wandering phase of stable fly larvae. In addition, the timing of wandering and



pupariation was examined in relation to photoperiod.

## MATERIALS AND METHODS

## Methods Common to Experiments

Stable flies were reared at  $25 \pm 2^\circ \text{C}$ ,  $70 \pm 10\%$  RH, and 18:6 (L:D) photoperiod in a vermiculite, wheat bran, fishmeal medium. This medium was used as the substrate in all experiments unless indicated otherwise. For all experiments, wandering larvae were collected from the corners of the rearing pans (30 x 40) prior to pupariation. As stable flies near the end of their larval development, they purge their gut, initiating the start of larval wandering. As larvae begin to wander, their activity level increases, causing them to follow the edges of the rearing pan and collect in the corners. Preliminary studies demonstrated that these larvae pupariated within 48 h. These larvae are referred to as wandering larvae in this paper.

## Determination of the Length of the Wandering Phase

Two sampling methods were used to determine the length of the wandering phase. An empty gut was used to note the initiation of wandering. Media in three pans were treated with 10 g  $\text{BaSO}_4$  (method used by Green 1964), one pan was treated with 20 g  $\text{BaSO}_4$  and one pan remained untreated as a control. To monitor for gut clearing, larvae were x-rayed using a General Electric grain inspection x-ray diagnostic unit. The  $\text{BaSO}_4$  could be observed in the gut until the larvae had cleared the gut just prior to the wandering phase. The first experiment used a destructive sampling method on a population of 600 larvae to esti-

mate initiation and termination of the wandering phase for the population. However, length of the wandering phase for individual larvae was confounded with the fact that the original 600 larvae were not all in the same physiological stage; hence this technique measures variation in wandering phase length for the population in this experiment, not for individuals. Five black teflon coated baking pans (11 x 21 cm) were each filled with 1 l of prepared standard stable fly larval rearing media. Late second-, early third- instar larvae were removed from separate rearing pans and introduced into the experimental pans (600/pan). The treatments were monitored for larval activity at intervals until evidence of wandering behavior was noted (ie when larvae began to accumulate in the corners of the pan). Four or five larvae were removed initially every 4 hr to monitor for gut clearing. The end point of pupariation was noted at pupal stage 4. The pupal stages are described in the Results section. The wandering phase length was calculated by subtracting the estimated time at which gut-clearing occurred from the estimated time at which pupariation had ended. Five larvae from each of three different positions were removed from each pan. These positions in the pan were, bottom front left corner, against the side of the pan, and in the center (not touching sides). A total of 15 larvae were removed from each of three pans every two hours until all larvae had pupariated (or until the supply of 600 larvae was exhausted). An assessment of gut contents was made by x-raying each set of 15 larvae at 20 kv, 5 mA for 2 min. Negatives were examined for presence of  $\text{BaSO}_4$  indicating food in the gut.

The second method used to determine wandering phase length was a constructive sampling method. This technique directly measured the wandering phase length for 40 individual larvae. Forty larvae (third- or fourth- instar) from the previous experiment were sampled to determine the length of the wandering phase for individual larvae. Larvae were kept in individually marked vials with media containing  $\text{BaSO}_4$  and allowed to feed. Larvae were x-rayed every two hours at 20 kv, 7 mA for 20 seconds. Because the larvae were alive and moving, they had to be x-rayed for a shorter time period than in the previous experiment. Twenty of these larvae were placed between two pieces of acetate and immobilized by securing the acetate with several paper clips prior to x-raying. The remaining twenty larvae were placed on top of an acetate sheet and subjected to freezing temperatures for 10-15 seconds prior to x-raying. In addition to the x-rays, larvae were observed every two hours to record the initiation of pupariation. Pupariation was recorded at pupal stage 4 based on the scheme of larval stages described in the Results section. Means and standard deviations were calculated for the twenty insects used for each x-raying technique.

### Effect of 3 Parameters on Length of Wandering Phase, Survival, and Pupal Weights

Three levels each of moisture, temperature, and light were combined in a factorial treatment design to examine their effect on length of the wandering phase, survival to the pupal stage, percent emergence, pupal wet weight, pupal dry

weight, and pupal moisture content. For this, forty wandering larvae were placed in small plastic containers with 200 ml wet vermiculite at one of three moisture levels, 12.3, 65, or 84%. Three separate environmental chambers were set at 20, 26, and 32° C. All the containers were covered by cloth. Light intensity was varied by wrapping containers with aluminum foil (0 lux); with six layers of cheese cloth to provide moderate light level (150 lux); and with one layer of cloth for the high treatment (700 lux). Containers were removed from their environmental chambers every four hr, contents were spread out onto a tray, and number of pupae in the 5-7 pupal stages were counted. The 0 lux treatments were taken into a dark room and inspected under a red safety lamp.

Twenty four hours after all the insects had pupariated, ten pupae from each treatment were analyzed for wet weight, dry weight, and pupal moisture content. The remaining pupae were allowed to emerge and percentage emergence was calculated. Percent survival to the pupal stage was also calculated and differs from percentage emergence in that it includes larvae that did not survive to pupariation, while percentage emergence is based solely on the number of pupae that emerged to adults. A factorial analysis was conducted on all factors. Data on the length of wandering phase were fit to a non-linear model (SAS NLIN, SAS 1987) which generated a mean and standard deviation for the pupariation rate curve. The mean corresponds to time at which 50% pupariation has occurred, while the standard deviation is an accurate measure of the slope of the curve, and

it measures essentially the speed at which the entire population pupariates. An ANOVA was conducted on both mean and standard deviation. The multiples comparisons (Fisher's Protected Least Significant Difference tests) which followed the ANOVA were considered to be significant if  $P = 0.05$ .

#### Effects of Density on Pupariation Rate

In order to assess the effect of larval density on pupariation rate, wandering larvae were arranged individually in test tubes and in groups of 50, 100, and 200 per 200 ml wet vermiculite. Densities were replicated three times. Three separate groups of 50 individual larvae were also used in the study. These larvae were placed in test tubes which were kept in a test tube rack. All larvae were kept in containers of vermiculite (ca. 70% moisture content) in an environmental chamber at 26 °C and with a 16:8 light:dark cycle. Containers were removed from their environmental chambers every 2-3 hours, contents were spread out onto a tray, and number of pupae in the 5-7 pupal stages were counted. The individual larvae could be observed without disturbing the test tubes. A pupariation rate curve was generated for each replicate. To calculate a rate curve for individual larvae the time to pupariation was combined across replicates. These data were fitted in a non-linear model (SAS NLIN, SAS 1987) which generated a mean and standard deviation for each replicate. The mean represents the time at which 50% pupariation has occurred, while the standard deviation is a measure of the slope of the curve. An ANOVA was conducted on the mean and standard deviation. The



mean time to pupariation and rate of pupariation of another density level (50 larvae) were also compared to the individual larvae.

### The Timing of Puparium Formation

Wandering larvae were placed in containers for observation under standard rearing conditions. Three groups of larvae between 385 and 546 individuals were used. The three graphs represent 3 replicates of wandering larvae which were removed from the rearing pans and placed in separated containers to observe the timing of pupariation. Containers were analyzed in the same manner as the previous experiment. The number of pupae was plotted over time.

## RESULTS

### I. Description of the Stages During Pupariation

A scheme of pupariation was developed to describe each stage of pupariation from the beginning of larval wandering to the end of tanning (Fig 1). These stages were used to delineate end points of wandering behavior. The preliminary studies involved 10 insects under normal rearing conditions. A stage 1 (the crawling stage) was an active, wandering larval stage, with no appearance of shortening. The stage 2 (reversible anterior retraction stage) was defined as when the larva began to shorten with its' mouthparts still visibly everted. This stage was reversible in that the larva would lengthen (resembling a normal larva) and attempted to crawl away when disturbed. A stage 3 larva (irreversible anterior retraction stage)

looked similar to the stage 2 larva, however it could not lengthen itself to resemble a normal larva when disturbed. Stage 4 larvae (barreling stage, as in Finlayson's (1967) description) were described as being oval, the same shape as normal pupae, with the mouthparts inverted, but with no visible tanning; this stage was pale white, nearly the same color as the larvae. Stages 5 (hardening stage), and 6 (darkening stage) resembled one another in morphology, but differed in color. Stage 5 was the hardening stage accompanied by the beginning of visible tanning, and was a light tan, while stage 6 was a darker version, a light orange-brown to dark brown in color.

The time between stage 2 and 3 was ca. 90 min, between stage 3 and 4 was ca. 60 min, between 4 and 5 was ca. 95 min, between 5 and 6 was ca. 27 min, and between 6 and 7 was ca. 125 min. The entire process lasted a total of ca 6.5 h. These times are approximate because these changes occurred slowly, and the process of pupariation was continuous.

#### Determination of Wandering Length

There was a high degree of variation in the wandering length of a population of 600 larvae as determined by destructive sampling (Fig. 2). The wandering length for 20 larvae determined by constructive sampling was  $10.6 \pm 3.5$  hrs.

#### Effect of Three Parameters on Length of Wandering Phase, Survival, and Pupal Weights

In the 3 x 3 x 3 factorial experiment, moisture content had a significant effect on fresh weight ( $P = 0.0509$ ;  $df = 2, 5$ ;  $F = 5.73$ ); pupae in media with higher moisture contents (67 and 84%) had higher fresh weights (12.16, 11.96 mg, respectively) than in media with 17% moisture content (11.14 mg). There was no significant difference in pupal fresh weights of larvae reared on media between 67 and 84% moisture. Pupal moisture content was also significantly affected by moisture treatments ( $P = 0.0001$ ;  $df = 2, 22$ ;  $F = 14.70$ ). Percent moisture of pupae in media at 67% moisture content (73.41%) was not significantly different than percent moisture of pupae at 84% (73.72%). These two values were both significantly higher than percent moisture of pupae at 17% moisture (71.36%). There were no significant effects of any measured parameter on dry weight of pupae. Percent emergence at 17 and 67% moisture was highest, but not significantly different between these two levels (88.5 and 88.1, respectively). Percent emergence at 84% moisture was 77.0, significantly lower than at the other two moisture levels. There was a significant interaction between the effects of moisture and temperature on survival ( $P = 0.037$ ; Fig 3). Only at high moisture content (89%) and high temperature (32° C) was there a dramatic decrease in survival.

The mean time to 50% pupariation was longest at the 84% moisture level ( $P < 0.0324$ ), but not significantly different at 17% and 67% moisture levels. The mean pupariation time significantly decreased in hours with an increase in tem-

perature ( $p < 0.0554$ ; Fig 4). There was an interaction between light and moisture relative to their effects on rate of pupariation ( $P < 0.0637$ ; Fig 5). Rate of pupariation was fastest at 67% moisture, slower at 17%, and significantly slowest still at 84% (Fig 6). However, this effect was only seen at the moderate light level (shade). Dry weight and % moisture were unaffected by the three parameters. Group effects were found on fresh weight, dry weight, and mean (all  $P < 0.05$ ). Table 1. shows the  $P$  values for all three factors and their interactions.

#### Effects of Density on Rate of Pupariation and Mean Time to 50% Pupariation

Rate of pupariation and mean time to 50% pupariation were not significantly different for groups of 100 and 200 larvae/200 ml wet vermiculite and individual larvae (rate:  $P = \text{n.s.}$ ;  $df = 2, 6$ ;  $F = 2.10$ , mean:  $P = \text{n.s.}$ ;  $df = 2, 6$ ;  $F = 0.88$ ). Mean time to 50% pupariation measures the average time that the population took to pupariate. Rate of pupariation measures the slope of the pupariation curve, or how fast the entire population pupariated. Rate of pupariation and mean time to 50% pupariation were also not significantly different for smaller groups of 50 compared to individual larvae (rate:  $P = \text{n.s.}$ ;  $df = 1, 4$ ;  $F = 0.01$ , mean:  $P = \text{n.s.}$ ;  $df = 1, 4$ ;  $F = 3.05$ ).

#### The Timing of Puparium Formation

The three graphs in Fig 7 represent three replicates of mixed-age groups of wandering larvae. The total numbers of larvae represented in graphs 1-3 are 439, 385, and 546, respectively. Puparium formation appeared to be random under the

16:8 light:dark photoperiod, showing no obvious circadian rhythms.

## DISCUSSION

The length of the wandering phase of *S. calcitrans* has not been previously studied. The wandering phase length is defined as the period of time from the initiation of wandering (typically characterized by gut purging) and the beginning of puparium formation. In flies, gut-purging is followed by an increase in locomotory activity and often the insect becomes negatively phototactic (Zdarek 1985). The timing of each phase within pupariation for *S. calcitrans* was comparable with that of *Sarcophaga bullata* as defined by Zdarek (1980), and Zdarek and Fraenkel (1972). The difference between the two dipterans was that *S. bullata* entered a 'red-spiracle phase' and *Stomoxys calcitrans* did not. The time from initiation of antero-posterior retraction to beginning of tanning lasted longer (four h) in *S. calcitrans*, than in *Sarcophaga bullata*, which lasted one h (Zdarek and Fraenkel 1972), or 3-4 h according to Zdarek (1980). Overall pupariation time estimates for *S. bullata* were 2.8-3.8 h (Zdarek 1980), and 5.8-6.8 h (Zdarek and Fraenkel 1972). For *Stomoxys calcitrans* the process of pupariation was estimated at 6.5 h. In addition, the descriptions of stages of puparium formation which appear in this paper for *S. calcitrans* quite closely resembled descriptions for the tsetse fly, *Glossina morsitans* (Finlayson 1967).

The length of the wandering phase for other Diptera is quite variable both



within and between species. The wandering length for *S. calcitrans* was estimated in this paper to be  $10.6 \pm 3.5$  h. Finlayson (1967) reported that *G. morsitans* had wandering lengths in the range of 1.4 h to 6 h, the latter time being rare. Under dark conditions, time from beginning of wandering to the barrel stage for *G. morsitans* was as short as 23 min. *G. morsitans*, however are quite different than other Diptera in that larvae feed from milk glands in the adult uterus, and are laid after the larvae have completed feeding (Finlayson 1967). Essentially they are laid at the beginning of their wandering phase, and they begin to burrow into the ground immediately (Finlayson 1967). *Sarcophaga bullata* spent 40-60 h in the wandering stage (Zdarek 1985), considerably longer than in *Stomoxys calcitrans*. Denlinger (1972) claimed that non-diapausing *Sarcophaga bullata* wandered for 24-48 h, but that diapausing pupae spent over two weeks in the wandering phase. The wandering phase of *Stomoxys calcitrans* is relatively shorter than most dipterans that have been reported in the literature with the exception of *G. morsitans*.

The wandering phase length of a population of 600 larvae was quite variable. This variability appeared to be, in general, greater than the variability in wandering lengths demonstrated in other Diptera. However, the methods of defining and measuring the wandering lengths varied among researchers, and the lengths may not be directly comparable. Other Diptera usually showed a higher degree of variability only when subjected to adverse environmental conditions (i.e. *G. morsitans* in the dark; Finlayson 1967). The length of the wandering phase in *S. calcitrans*



was also affected by environmental conditions. Mean time to 50% pupariation, and the rate of pupariation indicate two separate measurements of the process of puparium formation. This distinction has not always been clear previously in the literature. The mean time to 50% pupariation measures the average time that the population has taken to pupariate. The rate of pupariation, however, measures a different aspect of the pupariation process. It measures the slope of the pupariation curve. This essentially measures how fast the entire populations pupariated. For example, a population that had a slow rate of pupariation (a small slope) would pupariate slower than a population with a faster rate. Also, the population with the slow rate would have more variation in terms of the times that individuals begin pupariating. Mean time to pupariation was significantly greatest in media containing the highest moisture level (84%) over 17 and 67% moisture levels.

At one light level there was a highly significant difference in the rates of pupariation at the three moisture levels. Insects pupariated fastest at the moderate moisture level (67%), then slower at 17% moisture, and much slower at 84% moisture. This supports the hypothesis that insects pupariated fastest under favorable conditions, since survival to pupal stage was also significantly higher for insects in media at 67% moisture. Pupal fresh weight was also highest at 67% moisture. It was demonstrated in chapter 1 that wandering larvae of *S. calcitrans* consistently selected media at 67% over media at 17 and 84% moisture. All these

factors combined clearly demonstrated that 67% moisture represented the most favorable conditions of the three moisture levels tested. Although the effect of moisture on rate of pupariation was only observed at one light level, the differences in rate were highly significant and should not be ignored. The effect of moisture on mean time to pupariation was, however, a significant effect and clearly demonstrated the relationship between unfavorable moisture conditions and delayed pupariation.

Differences observed in the rate of pupariation may quantify plasticity in the system better than a difference in mean time to 50% pupariation. However, a shift in mean time to pupariation due to unfavorable environmental conditions is probably more likely and common, because of the fact that the population responds as a whole. For example, temperature had an effect on mean time to 50% pupariation and not on rate of pupariation. The mean time to 50% significantly decreased with an increase in temperature (Fig 4). This effect was presumably due to an increase in the metabolic rate resulting in faster development at high temperatures. This effect does not apply to my previous hypothesis that insects have a shorter wandering length under optimal conditions. In fact, the data on effects of temperature seem to contradict this hypothesis. Insects pupariated sooner at 32.5° C, then at 26° C, and lastly at 21° C, yet insects at 32.5° C showed the lowest survival to pupal stage. In chapter 1 it was demonstrated that wandering larvae selected media between 25-27° C in which to pupariate. It is

most likely that these data do not contradict the hypothesis that insects pupariate sooner or faster under optimal conditions. Instead, this effect is more simply due to an increase in metabolic processes resulting in decreased development time. However, moisture appears to cause a difference in the rate of pupariation. The curve is actually extended, where some insects begin to pupariate earlier than normal, and, in the same population, some insects end the process of pupariation later than normal (normal would be the pupariation curve at 67% moisture). This effect can be due to two phenomena. Once the population begins to wander, larvae are either beginning the process of pupariation significantly later, and/or some larvae are extending the wandering phase (delaying pupariation) significantly longer than normal. When there is a difference in rate of pupariation, this exhibits more variation among individuals and this also tells more about the behavior of the individual larvae. Given the evidence on site selection, and survival in different moistures, these data seem to indicate that individual larvae are manipulating the length of the wandering phase under unfavorable conditions (high moisture).

Insect hormones are likely to be involved in the delay of pupariation. Berreux et al. (1979b) demonstrated that in *Drosophila melanogaster*, a small peak of ecdysteroid existed in the haemolymph prior to a larger peak associated with puparium formation. Berreux et al. (1979b) suggested that this smaller peak was involved in the timing of pupariation. Under adverse conditions, the small peak as well as the larger peak are delayed (Berreux et al. (1979b). The secretion of

ecdysteroid is suggested to be caused in some way by favorable surroundings (Ohtaki 1966; Zdarek and Fraenkel 1970). Zdarek and Fraenkel (1970) demonstrated that *Sarcophaga argyrostoma* became more sensitive to ecdysone injections the longer they had been exposed to dry (favorable) conditions after being subjected to high moisture (unfavorable) conditions. It is not known how labile the system might be after the small peak of ecdysteroid (implicated in the timing of pupariation by Berreur et al. 1979b) is released by the insect. This may impose limits on the length of wandering, and on the ability of the larvae to choose a suitable site in that window of time.

A delay in pupariation under conditions of high moisture occurs in *Sarcophaga bullata* (Zdarek and Fraenkel 1970), *Aldrichina grahami* and *Calliphora lata*, *Sarcophaga peregrina* (Ohtaki 1966), and *S. argyrostoma* (Saunders 1975). Pupariation in *S. peregrina* was delayed over 100 h when the larvae were in wet rearing conditions (Ohtaki 1966). These findings demonstrated tremendous plasticity in the initiation of puparium formation. Ohtaki (1966) claimed that *S. peregrina* remained as "mature larvae for ten days or more without pupation." From this description, it is difficult to differentiate between the length of the larval period from the actual length of the wandering phase. Many of these terms are poorly defined in the literature. *Stomoxys calcitrans* seems to be capable of greatly delaying pupariation under adverse conditions such as high moistures, however survival under these conditions is very low (Hoffman 1968).

Removing *Sarcophaga argyrostoma* larvae from their food source 3-4 days early shortened the mean length of larval development (Saunders 1975). In the factorial experiment, *Stomoxys calcitrans* larvae were removed from their food prior to pupariation and placed into the relatively inert media (vermiculite). This may have affected the length of the wandering phase in these experiments, but it is unlikely because larvae were removed which were aggregated in the corners of the pan, indicating that they had already begun wandering. In addition, Fig 7 illustrates that larvae were removed only 2-4 h before the first few insects began to pupariate. In graphs 1 and 3 in Fig 7, at least half of the insects pupariated within ca. 10 h, indicating that they were not removed from their food prematurely (wandering phase length was estimated to be 10.6 h). However, the remaining 50% of larvae, and most of the larvae in graph 2 had been removed prematurely. Yet, the three graphs do not appear to be skewed to the right, indicating a delay in pupariation due to premature removal from the food source.

In this study, density had no effect on mean time to 50% pupariation or rate of pupariation. Large aggregations of stable fly larvae were not discovered in field sites (chapter 1). Gilbertson and Campbell (1986) reported during their evaluation of feedlots in Nebraska that stable fly larvae and pupae were never found clustered. However Parr (1962) claimed to have found large aggregations of stable fly larvae and pupae. Since large aggregations (>200 insects) were not found in the field, groups of 50, 100 and 200 should have been sufficiently large



to demonstrate an effect of density if one existed. It seems highly likely that groups of this size might form on occasion since female stable flies have been shown to lay eggs in close proximity to other ovipositing females (Bidgood 1980, Hoffman 1968). From this evidence, I would conclude that density had no effect on stable fly pupariation. Unfortunately, I did not measure pupal weights and survival; Zvereva (1986) discovered that house flies maintained an optimal density at which survival and pupal weights were higher in large groups than for individuals.

In experiments concerning density effects on mean time and rate of pupariation, there was a confounding effect brought on by the methodology of scoring pupariation. The individual stable fly larvae were kept in test tubes and were easily scored without disturbing their containers. Insects held in groups were poured onto a tray for scoring, thus being disturbed every 2-3 h. Groups of 25 *Calliphora erythrocephala* pupariated significantly later than did individual *C. erythrocephala* according to Berreur et al. 1979a; although the authors did not test for differences in the rate of pupariation, the slopes of the pupariation curves did not appear to differ. Berreur et al. 1979a also found that individual insects placed in rotating flasks in order to receive continual mechanical stimulation also delayed pupariation significantly compared to individuals that were not disturbed. The effects of stimulation were such that groups of undisturbed insects and individuals undergoing constant mechanical stimulation were not significantly different in their mean times to pupariation because the movement of larvae in the 'undis-



turbed' groups was enough to physically stimulate them (Berreur et al. 1979a). This indicates that the effect of density on pupariation was due to the mechanical stimulation by nearby larvae. However, Finlayson (1967) demonstrated that *G. morsitans* pupariated sooner in groups of ten placed in small one inch caps containing sand than single larvae placed in thin plastic tubing. Larvae in plastic tubing (with mechanical stimulation on all sides) pupariated sooner than larvae kept in small one inch caps (with much less mechanical stimulation). Finlayson (1967) found that mechanical stimulation seemed to decrease wandering length which contrasts the results from Berreur et al. 1979a. Regardless of the difference between the two systems, both mechanical stimulation and crowding affected pupariation in the same direction. In my study, *Stomoxys calcitrans* showed no differences in pupariation between groups of larvae which were disturbed frequently, and individual larvae which remained undisturbed. Since the effects of crowding and disturbance were in the same direction, it can be deduced that neither density nor disturbance (mechanical stimulation) had an effect on pupariation in *S. calcitrans*.

The initiation of puparium formation did not appear to occur as a gated rhythm (Fig 7) in *S. calcitrans*. The three graphs in Fig 7 demonstrate that the initiation of pupariation appears to be random with regard to the 16:8 light:dark photoperiod used throughout the experiment. However, this may be an artifact due to the fact that this population of flies has been in rearing for 5 years. Con-

sidering that the wandering phase lasted ca. 10.6 h, much of the wandering might occur in the dark, as it does in *Sarcophaga argyrostoma* (Richard et al. 1986). The advantages of wandering in the dark may be to avoid diurnal predators and parasites or to avoid desiccation during the day (Richard et al. 1986). The formation of puparia in *Sarcophaga argyrostoma* occurs at random during the light and dark periods (Richard et al. 1986), as was found for *Stomoxys calcitrans* in this paper. However, in *Sarcophaga argyrostoma*, the initiation of larval wandering occurred as a gated circadian rhythm (Richard et al. 1986). These differences may simply be due to the fact that *Sarcophaga argyrostoma* undergoes diapause, and *Stomoxys calcitrans* does not. Richard et al. 1986 found no correlation between the initiation of wandering and the initiation of puparium formation in the same insect. Hence, it may be incorrect to simply subtract 10.6 (the wandering phase length) from the time at which each insect had pupariated to estimate the beginning of wandering. When 10.6 is subtracted from the time pupariation begins, the initiation of wandering does not appear to be a gated event either. It seems likely, but somewhat speculative that wandering occurred mostly at night. However, if the experiment was done with longer nights, the pattern may have emerged. Richard et al. 1986 found that although *S. argyrostoma* showed a gated circadian rhythm under long night regimes, under short nights the circadian rhythm was less obvious, and under continuous light wandering was arrhythmic.

Zdarek (1985) hypothesized that delayed pupariation was an adaptation to

food scarcity. Zdarek (1985) determined that a minimum amount of feeding after the last instar larval molt was necessary for pupariation to occur in *Calliphora vomitoria*. The mechanisms for the larval-pupal molt are irreversibly activated at some time by this brief period of feeding. If larvae are undernourished, this post-feeding period is longer. The post-feeding period refers to the period after feeding stops and before the insect undergoes the behavioral switch to wandering. This post-feeding stage specifically refers to conditions of food scarcity, where a last instar larvae may not have fed sufficiently to reach an optimal weight. However, with no food available, it is advantageous for the insect to begin pupariation, because over time the insect may lose weight in a lengthened larval stage. This argument is analogous to my argument concerning extreme environmental conditions. Under extreme conditions such as flood or drought, there may be a post-feeding stage which is delayed by these conditions. For example, under drought conditions, pupariation is delayed while larvae are searching for a more suitable pupation site, yet eventually the insect will pupariate, as the pupal stage is known to conserve more water than the larval stage.

An important component to the extreme conditions hypothesis is the ability of the wandering larvae to travel some distance to search for more optimal conditions. In fact, although stable fly larvae were shown to select narrow ranges for a variety of environmental conditions (chapter 1), the extent at which this may be useful to the insect depends greatly on the ability of the wandering larvae to travel

relatively long distances. It was shown in chapter 1 that wandering *S. calcitrans* larvae could travel up to 9.1 m in the field, allowing the larvae sufficient time and distance to actually find a suitable pupation site. *G. morsitans* was found to travel from 0.4-1.9 m depending on the light conditions in the laboratory (Finlayson 1967), while *S. bullata* traveled more than 20 m when simulated by light (Zdarek 1985). This may or may not be sufficient to allow the larvae to 'choose' an optimal site from among the limited (spatially and temporally) sites available.

Stable flies showed a great deal of variability in wandering lengths of an even-aged population of 600 insects. With this initial variability to work from, the stable fly has, to some extent, evolved the ability to regulate (within limits) the length of its wandering length in response to adverse conditions. The variability itself, however, may also have arisen as an adaptation to a changing and unpredictable environment. The population of 600 larvae formed puparia for up to 75 h (achieving 95% pupariation). Within 75 h, weather conditions can change dramatically. It may be advantageous for an individual female insect to lay a batch of eggs which will pupariate at widely varying times. Since individual female stable flies lay from 80-120 eggs/batch (unreported data), the experimental population of 600 represents from 4-7 adult females. However, the variation in wandering lengths of larvae from individual females was not measured. The variation is more likely due to differences in food availability causing differences in larval development time.

This study demonstrated that there was relatively high variation in the timing of pupariation in a small population of stable fly larvae (600 insects). The estimated wandering phase length was  $10.6 \pm 3.5$  hours, which was relatively shorter than most dipterans that have been reported in the literature. The timing of pupariation did not occur as a gated rhythm under a 16:8 light:dark photoperiod. The initiation of wandering does not appear to be gated either, however this was not measured directly.

This study also suggested that stable fly larvae may be able to extend the wandering phase under extreme conditions (high moisture). This would allow the larvae more time to 'search' for more favorable conditions. Larval perception is limited to the insects immediate environment. The larvae can not 'search' for better conditions per se, but it may be able to extend its wandering phase making it more likely that the insect will crawl out of the excessive moisture into an area with less moisture. Hence it would be adaptive for the initiation of pupariation to depend on an external, environmental cue which would control the release of ecdysone, as Berreur (1979a) suggested. There are many documented cases of delayed pupariation in response to high moistures and other adverse conditions. The prolongation of pupariation may be an evolutionary adaptation to extreme environmental conditions, particularly those brought upon by floods and droughts.



Table 1. Significance levels (*P* values) in the factorial experiment with moisture, temperature, and light affecting various parameters of stable fly pupae.

	Moisture	Temperature	Light	Mois*Temp	Mois*Light	Temp*Light
Fresh weight	0.0509	*	*	*	*	*
Dry weight	*	*	*	*	*	*
Percent Moisture	0.0001	*	*	*	*	*
Percent Emergence	0.0614	*	*	*	*	*
Percent Survival	0.0062	0.0313	*	0.0373	*	*
Mean**	0.0324	0.0554	*	*	*	*
Rate of Pupariation	*	*	*	*	0.0632	*

\*not significant at the 0.1000 level

\*\*Mean time at which 50% pupariation has occurred



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Figure 1. Photograph of the successive stages in stable flies from the mature larval stage to the pupal stage.



**1**



**2**



**3**



**4**



**5**



**6**

Figure 2. Cumulative percent wandering larvae in a rearing pan of 600 stable fly larvae.

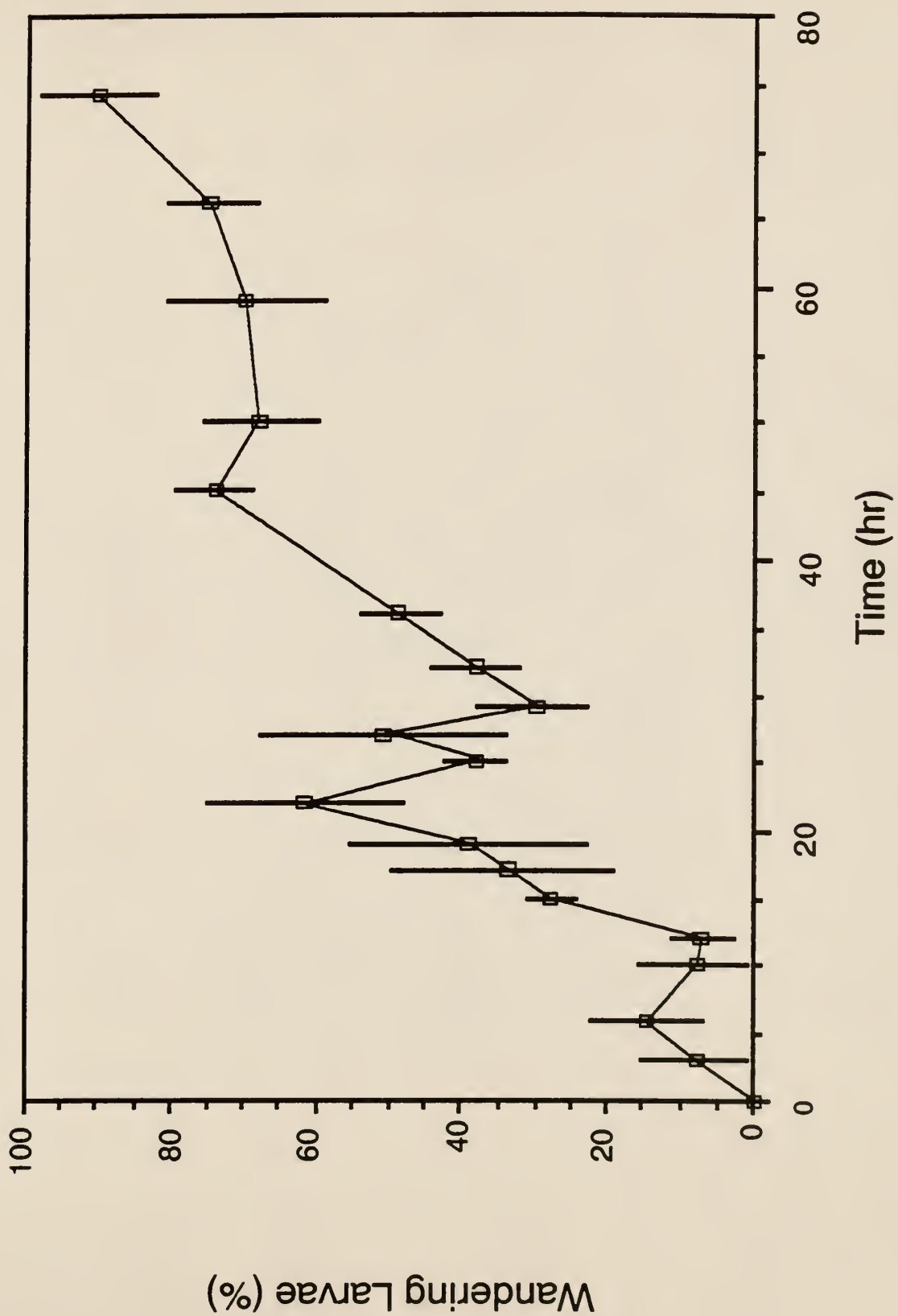




Figure 3. The interaction between percent moisture of media and temperature on percent survival of stable fly pupae.

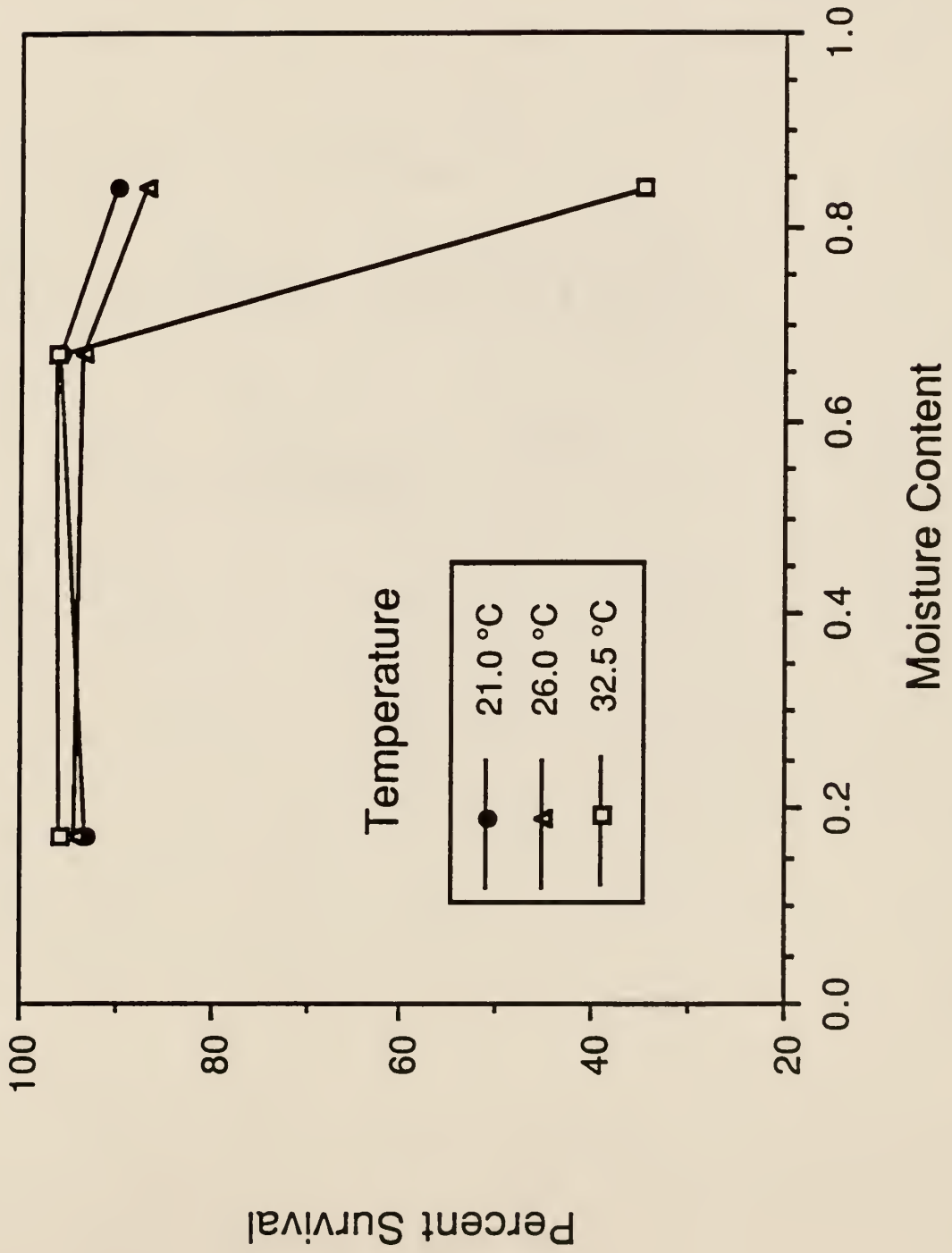


Figure 4. Cumulative percent pupariation of stable fly larvae at three temperatures.

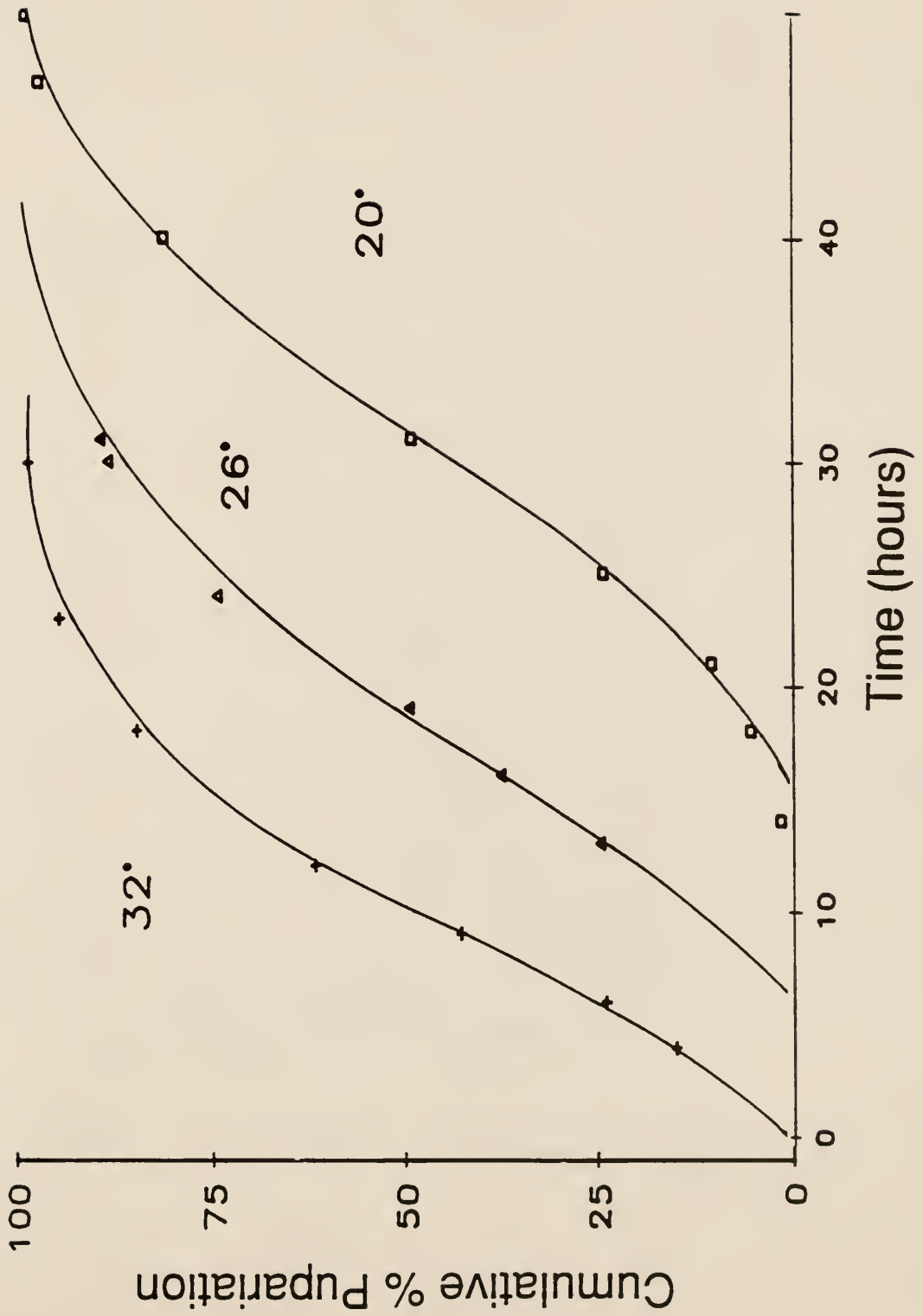


Figure 5. The interaction between light and moisture effects on rate of pupariation in stable fly larvae. The values 17, 67, and 87% indicate the % moisture of the media.

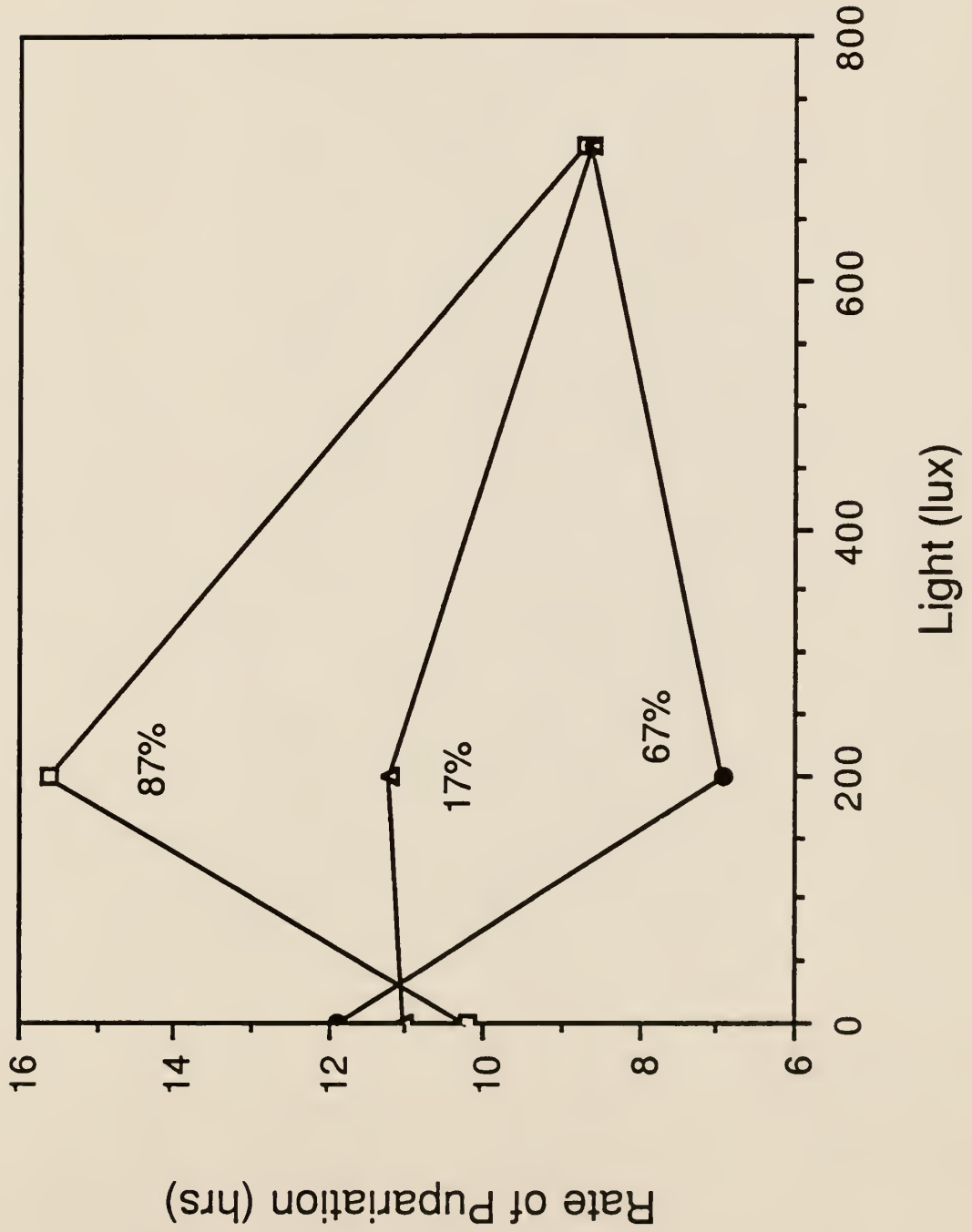




Figure 6. Cumulative percent pupariation of stable fly larvae at three moisture levels.

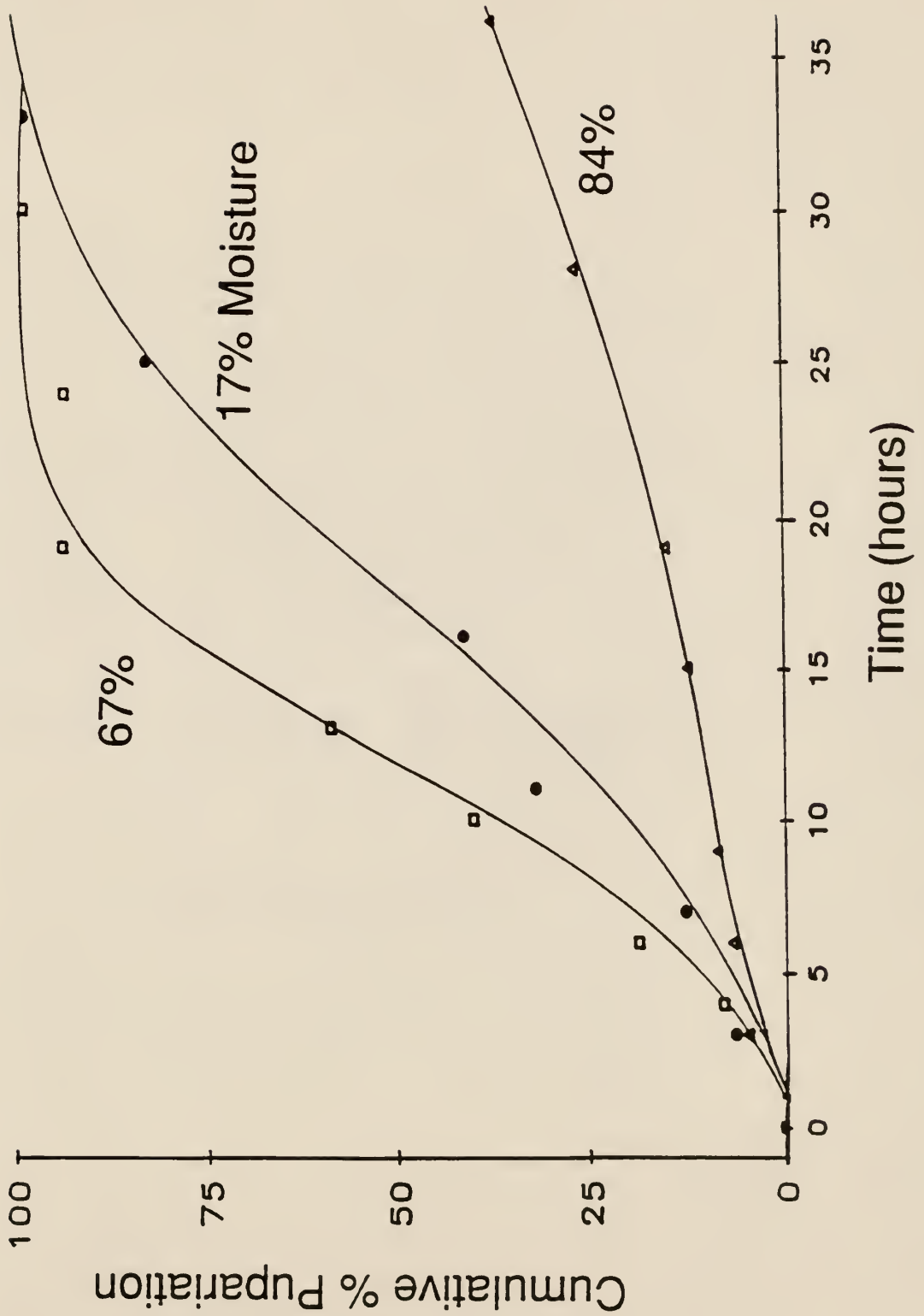
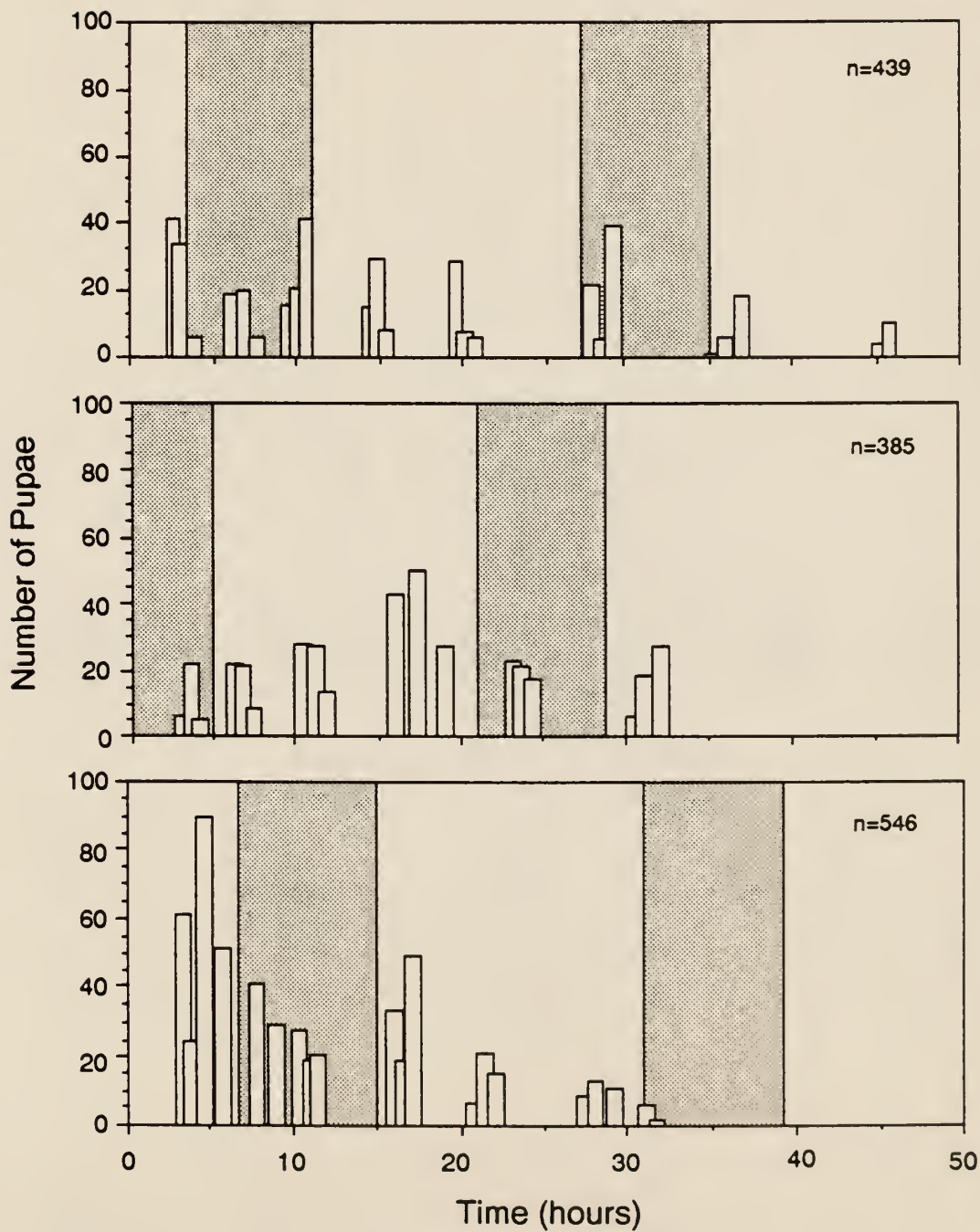


Figure 7. Number of stable fly larvae pupariating over time in three mixed aged groups under a 16:8 light:dark cycle.



## CONCLUSIONS

This study is unique in that it examines a number of parameters (moisture, temperature, light, pH, osmolality) and their effects on a wide array of traits such as, pupation site selection, pupal fresh weights, pupal dry weights, pupal survival, percent emergence, mean time to 50% pupariation, and rate of pupariation. By examining all these effects simultaneously in the stable fly, *Stomoxys calcitrans*, this allows a more comprehensive approach to environmental (chapter 1) as well as behavioral (chapter 2) components to pupation site selection.

In chapter 1, larvae selected a moderate moisture level (ca 68-71%) over high and low levels, while survival was highest in moderate to low moisture levels. High moisture seemed to be detrimental for survival and not selected by larvae in laboratory tests. This was slightly inconsistent with field results where larvae were found in the upper end of their range of total moisture sites available, meaning that they tended not to choose the drier sites in the feedlot. I believe that this response to high moistures is a response to potential drowning during conditions where the soil becomes water-logged. Also, if lower moisture levels had been used in the choice tests, I believe decreases in survival would have been observed due to effects of desiccation.

In chapter 2, high moisture contents also delayed pupariation, indicating that high moisture content was not as optimal as lower moisture levels. This is supported by the survival data in chapter 1. There was an interaction between

moisture and temperature; at high moisture and high temperature survival dramatically decreased. Thus, there may not be a simple straightforward relationship between pupation site selection and survival in different moisture contents, but the effect was quite large and it has also been demonstrated in a number of other dipterans.

In addition, moisture had an unusual effect on aggregation of pre-pupal larvae in uniform media (chapter 1). The extent of aggregation was higher in media at the moderate moisture level (69%) than at 35% or 82% moisture. This is interpreted as being consistent with the hypothesis that under sub-optimal conditions, insects will continue to search for more suitable conditions. At sub-optimal conditions (35% and 82% moisture) larvae do not have the chance to settle down into aggregations, as they do at 69% moisture. Once conspecific larvae are encountered, they may be inhibited from wandering further, since there is no need to disperse further to search for optimal moisture conditions. In the choice tests conducted with groups of 30 larvae, larvae were usually aggregated in the selected section of the pan. In less selected sections, larvae were generally more scattered, and grouped in small numbers. I would infer from this that larvae are being stimulated by environmental parameters first (i.e. by the suitable moisture level), then under 'selected' conditions, they are stimulated to aggregate in contiguous groupings with conspecifics. In one sense they are not a true group in that they form 'aggregations,' or 'collections,' defined by Immelmann (1980) as an orienta-



tion to similar environmental conditions, an accidental grouping with no particular relevance. However, stable fly larvae also appear to show 'true groups,' in that under uniform environmental conditions in the laboratory, larvae formed tight, contiguous aggregations, and seemed to actively seek out conspecifics. In the field, stable fly larvae and pupae were rarely found in aggregations in this study and by Gilbertson and Campbell (1986). An alternative explanation for the results found in uniform media is that the artificial nature of the pans caused larvae to accumulate in the corners simply by chance.

The effects of moisture on these various traits of stable fly pupariation are complex. There exist many inconsistencies in the effects of moisture as well as other parameters, indicating that pupation site selection is a complex process involving several interactions (moisture and temperature, moisture and density, environmental components and physiological components). The story is not clear from the present study, and more work needs to be done on the proximate and ultimate mechanisms of larval and pupal aggregation.

The effects of temperature were perhaps easier to explain. In chapter 1, stable fly larvae selected media of temperatures ranging from 24-28 °C. Percent survival to the pupal stage was highest in the moderate temperature of 26 °C (which falls in the selected range), although this trend was not significant. There was an increase in mean time to 50% pupariation with an increase in temperature. I believe this is simply due to an increase in metabolic rate.

pH and osmolality were found to be highly negatively correlated. Larvae consistently pupariated in high pH (9.3), and low osmolality (111 mmol/kg). Survival at the low osmolality was highest, and this data is consistent to the osmolality measurements of pupal sites in the field. Pupal survival was greatest at the second highest pH level (7.2). I would have expected survival to be greatest at the high pH (9.2), but at this pH survival was extremely low. This seems to be anomalous, because the media for immature rearing frequently reach this high pH level. Or perhaps, pupae are not adapted to such a high level, since pH greater than 9.0 is rarely found in the field.

Stable flies consistently pupariated in the dark, however, none of the other parameters were affected by light. This indicates that light may be a proximate mechanism which causes behavior which is adaptive in some other manner. Pupating away from the light, may lead the insect into areas that would avoid contact with predators and parasites, avoid competition with other species, or into areas with increased moisture to avoid desiccation. Many holometabolous larvae become negatively phototactic as they enter the wandering phase.

The discriminant analysis conducted on the four parameters measured in the field provided surprising results. The model claimed that pH was the most important variable in determining pupal sites versus non pupal sites. From the other studies in this paper, it is difficult to give any rank order to the variables, except to say that light (because of its little effect on all traits except in the choice test)

and perhaps temperature (because it appeared to vary temporally instead of spatially) are least important. There is evidence that low levels of pH may increase mortality of the face fly larvae (D'Amato et al. 1980, Grodowitz et al. 1987). This may be an important finding, since several studies have been unable to correlate larval breeding sites with pH (Rasmussen and Campbell 1981, Gilbertson and Campbell 1986). However, due to the lack of other evidence supporting pH as the most important variable, this result is suspect. This type of analysis is very reductionist, and does not allow for interactions between parameters, and alternative selection pressures on site selection, such as effect of conspecifics, competitors, predators, parasites, etc

The wandering phase of the pre-pupal larvae was found to be somewhat variable ( $10.6 \pm 3.5$  h). This is an important component to the site selection process. In order for the larvae to actively choose a site, the larvae must be able to manipulate the length of the wandering phase. There is considerable plasticity in the wandering phase length of the stable fly, indicating that choosing pupation sites may occur by delaying of the process of pupariation. Both the mean and rate of pupariation were delayed under high moistures, implying that high moisture levels are not suitable. In fact, survival was greatest in low to moderate moisture levels.

It was estimated in this study that pre-pupal larvae could disperse up to 911 cm, allowing the larvae to choose to potentially sample a number of different environmental conditions in the vicinity. However, there are limitations to the

insect's ability to choose temporally (within the 10.6 h of wandering), based on hormonal control of wandering; and there are limitations to choose spatially (within the maximum estimate of dispersal, 911 cm), based on the dispersal capability of the larvae in combination with what is available in the vicinity. The time spent wandering may not be sufficient to allow the larvae to choose an optimal (or even a suitable) site from among the spatially and temporally limited sites available. The wandering phase is hormonally controlled, and the hormone release may be influenced by the environment, thus allowing for some manipulation of the timing of pupariation due to environmental parameters (chapter 2).

Larvae do not 'search' for better conditions, but by continuation of movement they can bring themselves out of unfavorable conditions. Larval perception is limited to its immediate surroundings and can certainly not predict what other conditions are nearby. There is likely a window of time in which the larvae can accept a site, and this may be influenced by physiological state and external stimuli (chapter 2). Thus the larva is restricted to a site which it encounters during this window. There may be a critical minimum amount of activity by the larva before it can accept a site. It is possible that a larva is reacting to favorable conditions by positive klinokinesis (a change in rate or frequency of turns) or by positive orthokinesis (a change in speed). As a larva reaches favorable environments, it may increase the angle of turning which would keep the larvae in the favorable area. However this has not been well studied in stable fly larvae.

Although the choice tests in chapter 1 gave very definitive results, the proximate mechanisms involved in making the 'choice' are still not known. More work is needed at the individual insect level to determine mechanisms of movement and behavioral patterns during the wandering phase. It is important to understand the constraints on the system, to know what outer limits might be potentially reached by the larvae. There remain many questions to be answered, and some of the conclusions of this work are not definite. However, this study may lead to future work in examining environmental parameters and their effect on behavior and physiology of the stable fly.

PUPATION SITE SELECTION IN THE STABLE FLY, *Stomoxys calcitrans*

by

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## ABSTRACT.

The effect of moisture, temperature, light, pH, and osmolality on pupation site selection and survival of stable fly pupae, *Stomoxys calcitrans* L., was evaluated by means of choice tests. Of the moisture levels tested, 50, 68, 89%, number of pupae was highest at 68%. Larvae pupated in the dark when placed in a light gradient, and at a temperature of ca. 27° C., when placed in a temperature gradient. When given a choice of pH levels of 5.2, 6.4, 7.2, 9.3, stable flies pupated at the highest pH. Conversely, insects pupated at the lowest osmolality when given a choice of 111, 254, 403, 609 mmol/kg. Measurements of pH and osmolality of field habitats were found to be negatively correlated. A discriminant analysis of the parameters measured in the field indicated that pH was the most important variable in categorizing pupal sites versus non pupal sites. In texture choice tests, larvae pupated in rearing media containing sand over media without sand. In the laboratory, substrate density had an effect on the vertical distribution of pupae. In all density treatments, 82% of pupae were found in the top 4 cm in a 12 cm column. Distribution of pupae in uniform media was found to be non-random.

Field measurements of natural occurring pupation sites demonstrated that wandering larvae selected a broad range of the above habitat parameters in which to pupate compared to what was available in the field. Laboratory-reared larvae released in the field dispersed up to 911 cm, did not appear to relocate under more

specific conditions. In summary, the laboratory tested preferences of stable fly larvae were narrower than those observed in the field, implying that other selection pressures aside from those environmental parameters studied are important in the selection of pupation sites.

The mean wandering phase length for the stable fly was estimated to be  $10.6 \pm 3.5$  h. There was high variation in the wandering length of a population of 600 larvae reared in the laboratory. In a factorial experiment with three levels each of moisture, temperature and light, the mean time to 50% pupariation was delayed at high (84%) moisture levels relative to 17 and 67% moisture. The rate of pupariation was also highest at 84% moisture, but this effect was only seen in media with a moderate moisture level, 67%, and not at 17 or 84% moisture levels. Pupae in media with higher moisture contents had significantly higher fresh weights, while pupae in media with 67 and 84% had significantly higher moisture contents than at the lowest moisture, 17%. There were no effects of any of the three parameters on dry weight of pupae. Percent emergence was higher at 17 and 67 than at 84%. There was a significant interaction between the effects of moisture and temperature on survival; only at high moisture (89%) and high temperature (32° C) was there a dramatic decrease in survival. Density was demonstrated to have no effect on mean time to 50% pupariation or rate of pupariation when comparing groups of 50, 100, and 200 larvae per 200 ml wet vermiculite to insects held individually for pupariation. Puparium formation appeared to be random under a 16:8 light:dark

cycle, showing no apparent circadian rhythm.